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Burnet's Rickettsia Disease (Q Fever)

by
Prof. G. Maldolesi

Few problems have retained as much attention and interest and have had such rapid success in clinical, epidemiological and experimental research as during the last few years, the investigation of Burnet's rickettsia disease, (Q fever). In May of 1948 a series of papers were presented to the 54th Congress of the Society of Internal Medicine meeting at Karlsruhe. In October of 1949 on the topic of the relationship of pulmonary disease of virus and rickettsia, a paper was presented by Frugoni, Magrassi and Giunchi at the 50th Congress of the Italian Society of Internal Medicine meeting in Rome. On the 6th of May 1950 it was the subject of the Swiss Society of Internal Medicine meeting at Neuchatel; for the biological section the paper was presented by Mooser, for the clinical section by Gsell. Today our Society is meeting to consider this same question, especially because in these last few years, a great number of contributions in the field of clinical, epidemiological and experimental work have been made, not only in America and Australia, but also mainly in Europe; in view of these contributions it must be concluded today that this disease which up to some time ago was considered an exotic disease, also occurred during war

periods for causes strictly due to the war, especially it occurs following the movement of troops from one continent to another and now is being considered as a disease which is common in all the countries in Europe and occurs frequently in Italy, especially in some regions, in others it exists some of the time, especially during the time prior to the last war and exists in epidemic or endemic form.

The story of Q fever is short and recent. It was isolated and described for the first time in Australia by Derrick in 1935, as a special clinical form, the existence of which had however been noted among cattle workers in Queensland and was named fever of Mattatoi at the end of 1933, and finally was established in 1937 by Burnet and Freeman as a disease caused by a particular rickettsia, the name of which it now bears.

In 1928 Davis and Cox, studying a filtrable virus, which was pathogenic to the mouse and which, towards the end of 1926, Nogouki had isolated from a tick, the Dermatocenter Andersoni, identified in this pseudo virus a certain rickettsia, which besides being pathogenic for some animals in the laboratory, according to the observations of Dyer, demonstrated itself capable of provoking in man, a laboratory infection, the symptoms of which are similar to those of Q fever. Studied accurately in its morphological and biological characteristics, this particular rickettsia which had been described by Cox as diaporical rickettsia. The following year, however, Burnet and Freeman, Derrick and Dyer were able to ascertain

that no great difference from the immunological point of view existed between the diaporical rickettsia of Cox and the rickettsia described in Australia by Burnet, as an etiological agent of Q fever. This was demonstrated by Cox in 1940 in a brilliant manner, demonstrating the diaporical rickettsia was the etiological agent of the Q fever, the existence of which in North America was found out through the demonstration of a specific antibody of the circulating blood of the individuals located in various states, Montana, Idaho, Wyoming, Nebraska, Nevada, Arizona and Washington.

8 It was only during the last World War that the Q fever was first noted in Europe during the period from Nov. 1944 to July, 1945 among the American troops which were on the Italian front. In general, during this short lapse of time, were observed three small epidemic explosions, perfectly well geographically limited entirely to the military personnel. The first was observed among the troops which were on the Gothic line (Appennino, Tosco-Emiliano, Pagliano, Belvedere, Sassoleone), with a light continuation among certain spread out leave takers, successively, along the lake of Garda (Malcesine) and in Corsica (Rossius and Coll). The second epidemic center was born among the troops of the American Air Force, concentrated in the airport of Frottaglie in Puglia (Brumpt) and followed them during their home journey, both on a steamer in the Atlantic Ocean (Schultze), and successively, a short time after the landing in Camp Patrick Henry. The last center was represented by a laboratory epidemic, which was comprised of twenty cases of infection among the personnel in the

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military laboratory for the study of virus in Naples (Robbins, Rustigian and Bazucalupo).

Since in all three cases it was question of centers of origin and geographical distribution which were very well described, without any diffusion of the disease through the civilian population which was in direct and immediate contact with the military, it was thought that there was a possibility that the virus had been carried into Italy by the military from America or Australia, and that the infection had not spread among the civilian population or among the other troops because the imported disease had not found a favorable habitat in our country. Effectively on termination of the war, for a few years, no clues of any sort could be found in Italy of the existence of Q fever.

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In this respect one must note that during the years 1939 (Kalk and Frosenius; Stehr) 1941-1942 (Inhauser; Hirt and Bauer; Wieler; Bieling; Denning) in the Balkan Peninsula and especially in Bulgaria and eventually in meridional and eastern Greece, there occurred in epidemic form, a disease of rapidly diffusive character, characterized with high fever, with splenomegaly and with pulmonary infiltration with little clinical symptoms and identified only through radiological examination. This disease extensively affected the German occupation troops but it did in no way affect the native population, which proved itself non-receptive, almost as if it was

protected by an acquired immunity. The etiological problem of this form troubled Bieling as well as Erchberg who believed to have solved it by calling it a virus disease, "Balkan Grippa"; this dissection was questioned by clinics and especially by Imhauser who felt that it was not justified to identify as a common grippe, a disease which had such little diffusability.

The strain of this "Balkan Grippa" isolated in Greece by Caminopetros during an epidemic in 1943 and transported to America, were studied by the Commission for Acute Respiratory diseases at Fort Bragg and identified as a strain of rickettsia Burneti (Balkan strain). The identification of this was immediately illucidated and resulted in a large laboratory infection, which attacked the personnel of the institute for study of this disease, and was characterized by a symtomatology, clinical and serological, typical of that of Q fever.

It was only in 1946, that Gsell described the first cases of Q fever, definitely autochthonous in Europe, in Switzerland and in France (Strasbourg). The Swiss cases were rapidly made note worthy by the works of Gsell and Engel, Gutscher and Nufer, Wegmann, Wiesmann, Wisher, Roch, Alphonse and Loeffler, Wach. The disease appeared in the cantons (St.Gall, Argau, Granbunden, Ticino, Geneva, Vallais). In 1947 our first observations were made in Sicily and successively in Germany, in Martenberg, (Honi and Germer), in Baden (Bieling), simultaneously to a small

epidemic among American soldiers in Monaco (Denning) and lastly in 1946 in Asia (Imhauser). In 1948 the disease was described in Greece (Caminopetros) and Turkey (Payzin and Golem) in Italy in 1949, we had the small epidemics of Galesata (Giunchi) of Ravarino (Coppo, Bertoli and Zanussi), of Chianavalle (Babudieri, Bevere and Simonetti) and numerous cases in Sardinia (Magrassi, DeRitis and Scalfi) in Alto Adige (Forconi and Salvato), in Liguria (Bevere and Guerra). In the Abruzzi (Capurro and Gambacura), in the Marche (Petrignani and Bacchiocco; Marconi, Cristofanetti and Balico), in the Eolic Islands (Lopez), in the Republic of San Marino (Suzzi-Valli). The observations by De Prada, Parker, De Prada and Bell in Spain; by Funesca, Pinto Gauder, Azeveda and Lacerva, in Portugal; by Combiesco and Cull in Roumania; and finally by McCallum and Coll, by Manderson, by Harman, by Stoker, by Turner, by Adams and Coll, by Caugnet and Dudgeon in England.

From all this, it must be concluded that Q fever is a well diffused morbus entity throughout all Europe with the exception of the Scandinavian countries and Benelus and that in some of these countries it is quite frequent. Its presence has also been found in other places outside Europe and North America; in Morocco (Blanc, Martin and Maurice); in Algeria (LeGrand); in French Equatorial Africa (Giroud and Gaud); in the Congo (Jadin); in India and Palestine (Rosenkranz); in Central America it has been observed in Panama (Cheney and Geib, De Rodaniche).

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In North America, Q fever is a spontaneous disease and frequent in many regions, whether they be agricultural in nature (Texas, Illinois, California, North Carolina, South Carolina) or industrial (Michigan, Minnesota) and often appears in Connecticut with certain trades. The Official statistics of the USA point out thousands of cases of observed Q fever; but these numbers cannot express the actual real statistics of the disease since in many cases the symptoms pass unobserved and evade the statistics. In an experiment of Seck and Cole made in California with 10,000 individuals, who appeared to be healthy, there showed about 5% of cases of rickettsia Burnetii and a certain number of individuals showed an evidence of a state of disease or reminders of disease which might be referred to as Q fever.

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Therefore, in epidemiological study of this form of the disease one must consider the possibility which is not infrequent, of cases of infection which are not at all apparent.

With respect to Italy, the attention has been attracted by the paper of Frugoni and our paper at the 50th Congress of Internal Medicine and has shown that the observation of the cases has increased rapidly in the last six months. Actually no region of Italy can consider itself completely immune: Sicily, Marche, Lazio, Tuscany, Umbria seem to be actually the zones most susceptible right now.

Q fever can present itself at any moment or in any region

to the practicing doctor; thus he must be able to know it perfectly, not only from the clinical point of view but also from the epidemiological point of view so that he may be in a better position of recognizing it and diagnosing it rapidly, with assurance and decision.

Etiology.

The pathogenic agent of Q fever is that particular rickettsia isolated in Australia in 1937 by F.M. Burnet and in his honor named rickettsia Burnetii. Cox has proposed the name rickettsia diaporica, to remind us of the singular capacity of property that differs from that of other pathogenic rickettsia, known to date, in that it is filterable through candle; and Bergey has named it Coxiella Burnetii with the wish of separating in a particular group (coxielle) the rickettsia which have this property of filterability.

It is a very small organism of variable form and dimensions. It is observed in the cytoplasm of the cells under a form of micro colonies, which are distributed either uniformly or in masses at the periphery next to the cellular membranes in characteristic way from the center around the nucleus. It has the aspect of very small sticks of lanceolate shape or of form linked to diplococci. It is visible in chains of 2-3 elements, the dimensions vary from .25 microns by .4 microns in the lanceolate form, to .25 microns by 1 micron in the bipolar forms. It is immobile; it passes easily through the Berkefeld NW filter impermeable to the other common bacteria and to the other rickettsia, it passes through colloidal membranes with walls

of 400 millimicrons, impenetrable to other rickettsia. It does not pass through a single disk of Seitz (Cox).

The Burnet rickettsia, as opposed to all the other rickettsia, presents a major resistance to the common physical agents.

While the common rickettsia are easily inactivated by heat, drying, chemical disinfectants and ultra violet radiations, the Burnet resists to a heat of 60° for 1 hour and 30 minutes, to formalin in solution of 5%, to phenol in solution up to 1% and to 1 hour of ultra violet rays (Babadieri).

While the other rickettsia in organic material maintain themselves virulent for only a few days, the Burnet strain can make itself virulent for nine months and 18 days in blood, maintained at environmental temperature(normal). (Combesco and Dimitresco). According to Phillips, Burnet rickettsia maintained at ambient temperature proved to be infectious after 586 days; after six years they were still visible under the microscope and had conserved their antigenic power for immunization of the guinea pigs and to provoke the formation of antibodies noticeable during the deviation of the complement.

According to Jellison and Coll, rickettsia Burneti remains infectious for a long time, both in non-pasturized milk and in its derivatives, not obtained through boiling; for example, butter produced with infected milk, remains infectious after 3 months. Infected guinea pig blood, dried and left at ambient temperature,

may still be infectious after 162 days; urine after 45 days.

According to Smadel, Zabudieri and Bonciarelli the structure of Burnet's rickettsia does not appear any different than that of any other rickettsia under the electronic microscope.

In serological examination of Burnet's rickettsia, following spontaneous infection, does not give cross-immunity to any other type of rickettsia. The various strains of Burnet's rickettsia, known to date, give complete cross-immunity among themselves for experimental infection in the guinea pig; however, at the deviation of a complement, the various strains may demonstrate diverse quantitative behavior, especially with reference to the sensibility towards spontaneous infection of man. Smadel states that the antigen "nine mile" only gives a positive deviation of the complement and too late, meaning even 2-3 months after the beginning of the disease, while the antigen "Henzerling" gives a positive showing during the first days. The "Palermo" antigen, established by us starting with strains isolated from patients of endemic form, has shown itself even more precocious and in the beginning more sensitive. For such reasons the American department of Research has only used for suspected cases of Q fever, the antigen "Henzerling".

Experimental Burnet Rickettsia

A. In the cow.

The disease hardly ever presents any fever: the animal has an aspect of good health. The more frequently effects are those of mastitis, cheratite and in some cases, of bronchial pneumonia or pericarditis (Jellison and Coll).

B. In the guinea pig.

The subcutaneous, the intramuscular, the intraperitoneal methods have been used as avenues of inoculation in the guinea pig. With experimental infections Burnet's rickettsia, most of the time, causes a non-serious disease and of low mortality, it does increase considerably in cases where powerful infecting material is used, taken from a culture of rickettsia in chicken embryo, or from sputum from endomastous broncho pneumonia cases. The disease appears after a certain period of incubation, which varies according to the way in which it was introduced; from 3-6 days for subcutaneous infection, 4-7 days for intramuscular or intraperitoneal infection (Buttitta). The disease is always feverish(excepting certain sub-cutaneous cases) and with a clinical curve typically difasic, as it is usual in diseases of virus and rickettsia.

The formation of antibodies can always be determined or found in the infected guinea pig. If the subcutaneous inoculation was used, deviating antibodies can be noted after the 5th day, and agglutinated antibodies after the 15th day. If the subcutaneous or intraperitoneal injection has been used, the deviating antibodies occur after the 5th day and the agglutinated after the 10th(Buttitta). There is established a solid immunity which can be interpreted in a sense of a pre-immunization, given that, according to the observation of Parker and Steinhaus, the Burnet rickettsia in the experimentally infected guinea pig, persists in the organs for a long time after

the passing of the fever. It has, as a matter of fact been found in the liver after 60 days; in the testicles after 50 days; in the kidney cells and in the urine after 100 days; in the spleen 120 days from passing of the fever.

The anatomical, pathological alterations are represented as:

1. Constant index - has been noted an increase in the volume of the spleen; very rich in blood, lesions of the lymphatic glands, of small false myocardiac, which are all evident in the 5th day of the disease (Lillie).

2. Frequent index - in the lung it creates bronchitis and endovalvular exudations in the encefalo, circulatory alterations, which creates meningitis of the ph-rinx especially at the level of the bulb.

3. In the case of inoculations which have been done subcutaneously there is an inflammatory lesion at the point of ingress.

The histological alterations or symptoms were studies by Lillie and DaScavo. They are in general represented by:

Exudation, perivascular foci of the type lymphocidal, less frequently pyrophoblasts or monocytes, with vascular endoteliosis of the heart, lungs, the fatty tissue of the intestines, of the omentum, of the peritoneum, of the intestinal submucosa, of the epidermis, of the pelvis; less frequently in other regions(Lillie and Coll).

In the lungs there exists focal infections, with predominantly

monocytoid cells, while in the interalvular exudate, the epitaloid cells predominate. Similar follicular infections are encountered in the spleen and the liver where there are degenerative lesions of the parenchymal and regressive processes of the vacuolar type of the hepatic cells (Scavo).

In the brain is observed constantly a slight istiod movement around the lepto meninges with evident lymphocyte infiltrate and proliferation of the vascular endothelium. In respect to the nervous system there are signs of cerebral edema and presence of small foci of infiltration for the cellular way, irregularly spaced in the encephalitic region (Scavo).

Ways of Infection.

Under experimental conditions, rickettsia Burneti has been determined as a general infection in the animal or in man.

1. Through intradermic or subcutaneous inoculation. According to this method Blanc and Coll have observed infections in man.

a. A characteristic local lesion of the disease of diverse seriousness, it appears in the form of a boil or blister on the skin which persists for 10 to 12 days, or a scab which disappears in a few days.

b. The average or median of fever which appears in 24 hours and generally does not last more than 1 day, it can even be lacking.

c. Deviation of the positive complement, resulting in weakening.

2. Through subcutaneous injection (Caninopetros).

3. Through intramuscular injections in man, Blanc and Coll have observed following intradeltoid injections:

a. Locally a marked edema, persisting for almost a week and which does not tend to liquify and is accompanied by reaction in the region of the lymphatic glands or by leukocytosis.

b. High fever, up to 40°C. with a typically diphasic march. It is of diverse length of time and gravity in proportion to the quantity of injected virus, but it is well tolerated by the patient who does not need to be kept in bed.

c. Deviation of the complement and positive sera agglutination.

4. Through intra mammary injection. Caninopetros with the cow, the sheep and the goat has observed localized infections when he made the injection in the mammary lobes. In such cases the serological reactions (deviation of the complement, sera agglutination) are constantly positive according to Buttitts and Galle.

5. Peritoneal injection, (blood, urine, infected sputum). There results a typical feverish reaction with the relative formation of specific antibodies.

6. Through percutaneous absorption. Injection of suspension

of rickettsia through the intact skin in the guinea pig(Blanc and Coll). In man, Lickman describes an infection as a thing in the lung or going through the mucus membrane. Infections in the laboratory are known to have occurred through handling highly infectious material.

7. Through direct contact with the mucus membranes. Conjunctivitis nasally or rectally, infections are known that have been produced in the guinea pig by introduction of a thermometer covered with infectious material in the rectum.

8. Direct with the vaginal mucus membrane especially in copulation. Parker and Steinhaus have made copulation of virgin guinea pigs, non-infected, with infected males and rickettsia was observed in the seminal vessel. The females were infected up to 50% they aborted during fever, the fetus was infected. One of the three guinea pigs had an infection of such gravity that it died.

9. Direct inoculation of infected material through the excretory canals of the mammary glands, limited mastitis was determined in the corresponding mammary lobe.

10. Direct contact of the bronchial mucus or the alveolus epithelium of the lung. As is verified by the inhalation of finely powdered suspensions containing rickettsia powder(infectious material) (Blanc and Coll). In these cases of inhalation in addition to the fever there was also a typical pulmonary infiltrate of bronchial

pneumonia type. The cases of infection especially in the laboratory are numerous where the pathogenic character of this nature. This was verified in the laboratory of the OARD at Ft. Bragg, 1945, where all were infected with the exception of some who had the custom of wearing masks while working.

11. Through ingestion of infected material or foods.

This possibility is still controversial. Caminopetros has not seen such cases with sheep, goats or cows. Jellison and Back only have succeeded in infecting a bull calf, born of non-infected mother and feeding him with milk from an infected animal. They had not observed infection in the calf fed from a diseased cow which was suffering from a form of edimatic bronchial pulmonitis, with observed presence of rickettsia in the sputum. Gsell says that he has experimentally infected three demented persons by feeding them with infected milk. There exists in the literature on the part of some, the tendency, to try to explain the pathogenesis of certain epidemics by means of alimentary infections; mainly by ingestion of milk from infected animals, or from nutriment imbibed with excrements from animals carrying the disease, although they do not show the disease themselves. In the epidemic at Galleata, Girnchi proposes the hypothesis of the ingestion of comestible greens or other foods which were infected with excrements of infected animals. In the majority of cases these have been contradicted rather

strongly or put into doubt.

12. The clinical experience has not yet securely demonstrated the possibility of direct infections through contagion of touch from the diseased to the healthy (persons to animals) except in cases of handling of highly infectious materials, or excretions, as in diseases of pulmonitis, bronchitis, etc. (Moldolesi). Harman and Terzani affirm having seen doctors or nurses who had assisted, especially, wash dear people who have been diseased with pulmonary localizations of Q fever. These cases, however, of direct contact of man to man and which are in opposition to our knowledge of the epidemiology of rickettsia in general, eventually will be found to have had a definite reason. From what has been previously presented Q fever appears to be a disease of multiple means of infections; it must, however, be observed generally all the ways of infection, from the percutaneous to the intra-peritoneal and even to the ways of inhalation, always to lead to a general infection even in a latent form as is demonstrated from the rapid formation of antibodies from the sera reaction AF. Only through the inhalation, the most frequent, is there always a result with the formation of characteristic pulmonary infiltrate.

Vectors of Q fever which are Skin Ectoparasites.

As for all rickettsial diseases the problem of the existence of the vector of this disease of Q fever presents itself as to the eventual vector of the virus. For the endemic form has been indicated that ticks, in Morocco, Blanc and Coll speak of the

gerbillo.

It has not been systematically examined whether they are infected specific antibodies in the blood have not been demonstrated or revealed through sera agglutination or deviation of the complement. Autopsy has presented splenomegaly or other characteristic alterations of the moribund form. In any case we had injected in the peritoneum of the guinea pig "pappe di milza" and other organic elements taken up from suspected ratti; in these cases we concluded that there was an infection of Burnet's rickettsia. Similar result was obtained recently by Wiesmann in systemic ways as well as serologically and microbiologically.

It appears impossible, considering this, to prove a rickettsia burneti deposit in the Sicilian rat, comparable to that of the Australian bandicoot and the maroccan gerboa, a deposit which as with other rickettsia close the epidemiological link from virus to man.

As for the vector, it has been identified as the ticks for the Austrian strain, which can carry the infection from the bandicoot to man.

A systematic investigation carried out in Sicily with some infectious demonstrations has led to the consternation that in the "reggruppati animali" the infection of ticks was not demonstrated as being of a notable importance. Specimens of these ticks on sheep, bovines or infected donkeys and put from these onto guinea pigs, have not

been able to create the typical feverish reaction, nor to demonstrate the formation of specific antibodies, revealed through coagulation, agglutination, deviation of complement. These research works would exclude the possibility of the preexistence of Bennett's infection in these groups of animals that would have to be necessarily conditioned through the intervention of ticks or other ectoparasitic arthropods, functioning as the vector of the infection. Since then, Deen and Coll, Jollison and Coll, have arrived at an analogous conclusion through examining systematically the ticks which have been introduced deeply into the Orecchio of the cow (*Otobius Meunieri*) did not find infection above a certain minimal percentage, less than 2%; thus they could not attribute disease, the importance of the cause or factor, efficient or direct of the mechanism of the infection.

Even though the role of the parasite, especially of ticks, has a factor contributing to infection through which Q fever could be spread, represents maybe high coefficient of major importance and should not be excluded at first.

That the ticks can contain and maintain activity, the virus of Q fever has been demonstrated through the first experiment of Davis and Cox and of Parker and Davis, who have isolated for the first time in America, the disporical rickettsia credited to the tick: *Dermatocenter Anderson*. Furthermore, similar infections may

occur spontaneously in the tick, even though in small proportions and this has been demonstrated by the systematic investigations of Beck and Coll and of Parker and Coll, but in the majority of the common epidemics it must be recalled that their reserve of virus is partly constituted by the live ticks, but we must consider the efficiency factor.

On the other hand we must not disbelieve that because of the fact that some of our investigations on ticks in Sicily have had negative results, we are not authorized to exclude the fact that extending to a larger scale our investigations, it would not be possible to demonstrate that in Sicily a certain number of the ticks, even though not numerous, can be found infected with Burnet's rickettsia and we must not refuse at first the possibility that this eventually may not be very probable. In such a case, in the more common epidemics, the tick must function as a reservoir of virus and not as much as vector for propagating the infection, this is certain as to when it feeds its vectors it must necessarily become infected. So then the tick and the major part of the species not only conserves the rickettsia but can also transmit it according to hereditary mechanisms and thus results a closed circuit where there is stabilization being established, where the virus becomes securely conserved in a practically indefinite way.

Having been demonstrated that the virus remains active, even in a dead tick, (Blanc and Coll) or even in powder of ticks (Blanc and

Coll) and that experimentally it is sufficient to know that they transmit the infection to man, that there be direct contact with an infected tick through a non-altered skin(Liekmann and Coll) and furthermore the inhalation of powder, which has been ground (Blanc and Coll) it cannot be excluded that the possibility that powder can result in infection through the presence of residual of infected ticks. Many species of ticks have the habit of leaving the animals after having sucked the infected blood(in the Hyalomma Savignyi, which is common in Sicily in animals, throughout their life cycle), and to return to sawdust or to straw where they can deposit eggs and can make them infected. Generally the regions that can be easily infected can be recognized through the presence of dead ticks in sawdust, in straw or in general in the stable. Through these infectious media(p. uers, straw, sawdust, etc) it is possible that the virus can be transported at long distances and establish epidemics, according to the type which was found in the carpentry shop of Asrau(Gsell), during the working of the wood, which contained certain residues of ticks, through dispersion in the environment of infectious material.

It has been determined, even though only in the experimental way, that the ticks can infect and create diseased animals, even though resulting in forms that are not readily apparent; that once infected this constitutes a reserve of the virus which can not only not be lost, but can further continually result in infections

through means of the eggs and the feces and above all, can be transmitted indefinitely through hereditary means. It must be concluded that the ticks, even though it does not constitute the proper vector of the infection and of the disease to man, represents still the vector of the virus in so far as it can maintain infection in dust, wood, sawdust, straw and other materials, other than the feces and the eggs, even in the dry state. Considering the great resistance of Burnet's rickettsia to the common physical agents and especially to drying, we must conclude that the mechanism represents one of the factors which in practice, conditions the possibility of infection through inhalatory means, which represents the way most common to man and probably for animals.

In this respect one must not forget to mention the experiments of Shu-Hsian-Chao, who recently has demonstrated with respect to Prowazeki rickettsia, that the resistance of the rickettsia is conserved or maintained in organic materials (feces, organic remains, etc.) especially the resistance of the virulent strain, very notably in respect to particular factors, especially heat, humidity, etc.

In view of these factors, which can be modified today by climate or location, the activity of the virus conserved in powder form or in dust can be influenced in the case of Q fever, modifying

either in a negative or positive sense, its activity, and it seems that in most cases the dry state is the most favorable for the conservation of this virus.

Of the other factors, especially the factor tick, which has not been sufficiently illuminated through observations and experiments, there must be still remembered that, in so far as this constitutes one of the important mechanisms by which the individual infections can be stabilized, for example in the laboratory, as well as the epidemic infections (through dust inhalation of particles) and finally the factor "zecca" through which the factor tick which maintains the epidemic in a determined region.

Epidemiology.

The Burnet rickettsia in a infective disease common as well to man as to animal so that it really constitutes a true and proper "zoonosi".

In man it can present itself in diverse forms:

1. An epidemic form, which is the one that has been most often described until now, especially by anglo-saxon AA. and is easier to find in the city and in country towns.
2. An endemic form with a prominently rural character; its existence was first ascertained in Sicily and has been successively demonstrated by Deek and Coll in California. Its presence has been described in Greece by Caminopetros. With respect to the remainder of Italy, the presence of the focal infection of long duration has been established in the Republic of St. Marino, described by

Suzzi-Valli. From the information brought out in the meetings and discussions (DeCastro, Suzzi-Valli, Fontana) during the discussion that followed my presentation on Q fever in March, 1950 to the Medical Surgical Society of Romagna, it must be concluded that there exists a large endemic zone in Romagna, which extends deeply into the Tuscan-Emilian Appennines and which was securely established prior to the foci of military epidemics which appeared during the last war in 1944-45. It is not difficult to think that there exists other endemic foci of infection in other regions whether to the north or the south, in the mountains as in the valleys. It must exist in the animals, in the milk and in the meat, also in the local industries as well as in the saw mills and the carpentry shops. In this respect we must mention the disease with non-epidemic character which was described in Alto Adige by Forconi and Salvato.

A. The Endemic Q Fever.

According to our research in Sicily, the endemic Q fever is extensive in many parts of the island and the countryside, where it occurs as isolated, where were not demonstrated direct or visible bonds or contact either through simultaneous cases or through cases occurring previous to the disease. They are diversely scattered throughout the different seasons of the year, without any apparant variations of climate or temperature, without prevalence for either one of the sexes or being determined by either one. Contrary to the American cases, our cases have been observed having a preference for the female sex, with the exception of children and young girls. These cases have constantly demonstrated characteristics of affecting almost exclusively and always to

be in a determined zone where there existed a stall or a barn and the animals of which, even though they belong to different species, were demonstrated to be infected with this rickettsia. There thus exists for most cases of endemic Q fever, a constant relationship either topographical or due to work conditions, between man and animal, either endemic or zoonotic.

Our experience demonstrates that:

1. Wherever a new case of Q fever is encountered in the countryside, the zoological examination always indicates that in the immediate vicinity or in immediate conjunction with the case, there exists a barn or congregation of diseased animals, even if these are in non-apparent or non-active form.

2. If in a grange or a barn a diseased animal is found:

- a. A systematic investigation demonstrates easily that for other animals, even of different species, there are presented infections of these forms, even of non-apparent forms, even of the fact that they are in the same barn or stall.

- b. Around the central nucleus of this barn or grange at different times and different cases, it is possible to demonstrate from time to time a certain number of cases of these diseases to man. In our patients of rural endemic cases, are in general, herdsmen, employees of the barn, or housewives. One of our female patients was the wife of a man who carried the milk from the barn; the man did not show any disease even in the last

year that could be called Q fever, but he did show the constant deviation of complement. An analogous case has been described by Elemen. In our cases there also existed some who had no direct contact with barn or farms, but who for their travels through the countryside, used horses, mules or donkeys as transportation and who were thus affected by the inapparent form of Q fever.

In view of these veterinarian and clinical reports and taking into the contemporaneous approach when in a rural zone, there occur cases of absolutely sporadic infection of Q fever, with a single outbreak, whether it be numerous animals affected by the active form or whether they be affected by the inapparent disease, in view of all these, we initiated in 1947, a diligent and methodical work of research based on systematic serological examination and when there were any doubts, went into the biological tests, using guinea pigs. Our work received impulse and help in 1949, when we were able to benefit by the precious collaboration of Prof. Mirri and the large veterinarian material offered by the Sicilian Zooprophyllatic Institute that he directs. It was then possible to study individually a large number of focal endemic cases and of "endozootics" which comprised a large part of Sicily and the province of Palermo, Caltanissetta, Enna and sections of Messina, Catania, Trapani, Agrigento, Ragusa. In all this large agricultural region, whether it be in the valleys or in the mountains, this endemic form demonstrable in man and appearing in

close conjunction with an endozootic of domestic animals, the knowledge of clinical, epidemiological matters, as well as the geographical extent of the disease are of great importance for knowing the general epidemiology of Q fever.

The results obtained which will be presented later in this discussion have been successively confirmed by other authors, whether they be European or American.

Another method has been followed by Beck and Coll in their investigation in California. Instead of our clinical method, they have followed one which is characteristically statistical with research with "Tappeto" carpet(blanket?). In the first series of investigation these other authors have been able to establish the existence of approximately 300 cases of Q fever throughout all of southern California, but it was almost impossible in cases to establish any epidemic connection. Only evident was a prevalence for the male sex and for the individuals connected with the milk industry, the meat products, the canned meat and the skin products. It must be said that these occupations were those that obliged a direct and continuous contact with the animals or with the products of direct derivation and working with them in the rough stage.

We shall go now to the investigation of large numbers that these authors examined as to their reaction of deviation of complement in the blood of approximately 10,000 individuals who were apparently healthy but resided in places where cases of Q fever

would have been observed. A positive result was obtained in only 5% of the cases examined; but the positive cases were almost exclusively found in individuals who had lived for a long period of time in the areas suspected of endemic and prevalently in individuals who were close to the occupational work previously mentioned. Among the cases that gave positive results, a certain number was made up of individuals without objective signs and without information leading to the conclusion that there was an active Q fever infection or that there had been for some time. It is of value to say that they were probably carriers of an infection of the disease in an inapparent stage. Granting that very frequently the cases appeared to exist in relationship with animals or in professions dealing with animals or with their raw products, these authors thought that endemic could be established as related with this Burnet zoonosis, which previously that had demonstrated in that area and putting thus in evidence, cases of spontaneous Q fever in many species of animal. These authors then went on to examine systematically the bocines of that area, and they found that from 10% to 20% were infected, the majority of them with the inapparent form of the disease. The milk of those infected animals and the butter produced from it were demonstrated to be capable of resulting in a typical Burnet rickettsia disease in guinea pigs when injected in the peritoneum. These authors concluded by affirming that the animals were the source of the endemic in respect to the transfer

of the infection, ingestion of milk or butter has some importance, but the most important, was the direct contact with the animals and with the raw products derived from them (working with milk, meat products, skin or hides, and canning meat products).

B. The Burnet zoonosis.

Analagous results to those obtained presented in the publication of Beck and Coll in March, 1950, were obtained in our research which was initiated in 1947, and communicated in 1947 to the 50th Congress of the Italian Society of Internal Medicine and latter on, to the Society of Experimental Biology, section of Palermo on the 7 January, 1950, and in the presentation to the Sicilian Regional Congress of our Society in February, 1950.

Our investigations have demonstrated that Burnet's rickettsia presents itself in domestic animals in a disease characterized by high diffusion and which, originating in a barn or herd, can extend rapidly and massively to all the animals which are stabled in that barn or belong to that herd without any distinction to the species. In Sicily, it is a spontaneous disease of bovines, equines, sheep and dogs. In all these animals it almost always appears as an inapparent infection.

In the goat, Burnet's rickettsia, other than being an inapparent infection, can also give rise to a disease with a course very similar to brucellosis, resulting in abortion (Meldolesi and Kirri, Kilchsperger and Wiesmann, Caminopetros).

We have demonstrated the presence of Burnet's rickettsia in the milk of infected goats through the biological test but in contrast with the statement of Aminopetros, the animal demonstrate at the same time a positive deviation of the complement for the specific agglutination. Not all the goats with deviation of the complement of a positive sera agglutination, had infected milk; this confirms the experience of Parker and Coll, who, injecting directly into the mammary tissue some infecting material, could not demonstrate later rickettsia in the circulating blood, but have found in the sera specific revealing deviation of the complement and always to comparison with the rickettsia in the milk.

In Sicily Burnet's rickettsia is very widespread in goats, entire herds are struck. Systematic investigations by Mirri in Palermo county have demonstrated up 45% of infected goats among those examined. Lennette and Coll gave 43% infection for California.

In Sicily sheep have shown behavior similar to that of goats in respect to spontaneous infections of Q fever, whether it be with respect to the frequency of infection or of infected milk or through characteristics of the disease in those places. Also all the infected sheep showed deviation of the complement and positive sera agglutination and also they did not produce infected milk. But we have observed, as did Caminopetros, three cases forms of acute bronchial pulmonitis.

Also the sheep presented in Sicily of infected cases, according

to Mirri 47%; in California, Lannette and Coll found 37.9% infection.

Among the equines, horses, asses and mules, the infection in Sicily is frequent, usually completely inapparent. In no case did we find an equine with positive serological reaction where the objective examination furnished data for a previous or actual disease in apparent form. For the equines in respect to Q fever there does not exist now other data in the literature except for Caminopetros, the animals have only received the experimental infection.

In the bovines, rickettsia in Sicily is frequent but only in inapparent form. Besides ourselves, Wicsmann has succeeded in demonstrating rickettsia in the urine of apparently healthy cows.

Mirri, with his systematic investigation in the city and county of Palermo, has put into evidence a deviation of the positive complement of 33% in cows that were milking. Derrick and Coll in Australia have found positive results in 13 animals among 879 examined. In California, Shepard and Huebner have found positive 41 cases out of 150. Beck and Coll in 60% of the cases.

Huebner and Coll, having observed by means of biological tests in guinea pigs that the milk distribution by four dairies was infected with Q fever examined all the cows in that area and found that there was from 10% to 20% infection.

Jollison and Coll, having observed that a pregnant cow in apparent optimum physical condition, was eliminating infected milk,

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kept her under observation for four months, during which time the elimination of infected milk continued uninterrupted; after which they kill her. Microscopic examination evidenced no splenomegaly; large bodies through serocysts, pleuritic adhesences through a recent bronchial pulmonitis, a mastitis. Subjected to the biological test through different organisms, the results were negative by means of the fetus, the amniotic liquid and all the organs, with the exception of the mammary glands and the supramammary lymphnodes. With respect to these last, the presence of rickettsia was securely demonstrated. When the spleen, by injection unto guinea pigs, did not result in feverish reaction, but in one of four animals injected there was later a positive deviation of the complement. It was just possible to give a positive proof through the use of the spleen even though there was a lack of increase of volume.

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In all the infected herds, the herd dogs give us a positive deviation of the complement. By second investigation, ulterior to that of Mirri, showed a deviation of the positive complement in 50% of the dogs which had been captured by the municipal dog catchers of the city of Palermo. In one of these foci of acute broncho-pulmonitis was found, from which was isolated Durnot rickettsia in pure culture.

There does not exist any other information with respect to this animal in the literature; only Caminopetros, Blanc and Coll have written that these animals have recieved the experimental infection

of Burnet rickettsia.

With respect to other domestic animals which might show this spontaneous disease, they are not known at this time. Only Libicieri has found a goose with deviation of the positive complement. With respect to the wild animals, other than the Australian bandicoot, and the Moroccan gerbillo, there is record of some rats in Australia. and in the same place of the *tricolurus vulpecula* (Burnet and Freeman), in America, the porcupine, Rocky Mountain Laboratory. To those animals which have received experimental infection can be added; several species of rats and mice of house and country, the rabbit, domestic and wild, the ape, camel, sparrow, pidgeon, cock, parrot and the "scoiattolo" according to Caminopetros.

Of all the animals where we have observed positive negation of the sera complement, that is to say, signs of spontaneous Burnet's rickettsia whether accompanied or not with apparent disease, we have systematically attempted to carry the rickettsia into the guinea pig, the biological proof in the animal species according to inoculating once during;

- a. During the feverish disease, the blood.
- b. At the peak of the feverish disease, milk or urine.

In the case of the urine it can be demonstrated infective also for animals and it may take a long time to arrive from the apparent disease to a clinical recovery.

The fact that we have demonstrated that infected animals also continually eliminate rickettsia through the milk or the urine; the y demonstrated resistance of Burnet's rickettsia to all the

common physical agents (heat, exposure for a certain amount of time to ultraviolet radiation) seem to make it lose its infecting activity; all these can easily explain why the rural endemics once established go on maintaining their presence in infected animals; and also on the other hand the rural endozoosis is, in its turn maintained in the presence of these infected animals also can effect forms completely inapparent of infection and always in condition to eliminate as well for the animals as for man rickettsia through the urine and to contribute thus to maintain infected the soil, house, etc.

Once a location is infected, whoever lives there can be infected, by inhalation of the virus or directly through the intermediary of dust, the virus can also be acquired through the means of ingestion of milk or foods.

The mechanism as well of the established as of the persistence, whether it be the endozoosis or the endemic of Q fever becomes better understood through reciprocal interchange of the endozootic phenomena, infections of ticks and of the direct consequence of all these phenomena upon the conditions of the soil, the houses or the water supply, etc.

Endemic Q fever.

The study of Q fever epidemic is much more complex due to the large variety of the clinical framework and epidemiologic mechanisms.

The varieties of the epidemiologic mechanisms are related to:

1. The resistance of the virus reaction to time or to common physical agents and to its maintainance in liquid and in general in organic material.
2. The large number of the means of infection through percutaneous and permucosa way, through means of inhalation and finally through means of ingestion and the possibility of excluding direct contact with diseased people cannot be omitted.
3. The dissemination, especially in the endemic form, of the infection through animals to man with easy interchange and interdependence between endemics and zoonosis and the eventual development of epidemics.

Due to these variable interfering factors, in practice four types of epidemic mechanisms are possible:

1. The simplest and most evident mechanism is represented by the laboratory epidemics or surgical room epidemics, where the epidemic is obtained through encounter between the virus in its native state and the receptive individual; this mechanism is very, very simple.

There is no laboratory in which work has been conducted with Q fever which is immune to infection, regardless of the most dilligent prophylaxis measures.

We have had 7 infection cases among research and personnel of the microbiology laboratory of the Institute.

Laboratory infection cases have been reported with certain

frequency in the literature:

| Location | Date | Researcher | No. of cases |
|------------|------|---------------------|--------------|
| Washington | 1940 | Hornibrook & Coll | 15* |
| Fort Bragg | 1945 | CARD | 16 |
| Naples | 1945 | Robbins & Rustigian | 13 |
| Washington | 1947 | Huebner | 18 |
| Montana | 1948 | Oliphant & Parker | 2 |
| Montana | 1949 | Oliphant & Gordon | 6 |
| Hamburg | 1949 | Weyer | 11 |
| Monaco | 1949 | Kikuth & Bock | 25 |
| Stoccarda | 1949 | Hauck & Weyer | 7 |
| Panama | 1949 | DeRodaniche | 2 |
| Zurich | 1949 | Mosser | unknown |

* 1 dead

In addition to these cases of infection which can be traced back directly to the function of the laboratories, must also be considered as laboratory infections those cases which are observed among occasional visitors, as has happened in our Institute on two subjects who were infected through short visits to our laboratory of microbiology. Cases of this type are not rare in the literature. Cases of this type goes back to 1938 (Dyer) who had shown the first practical demonstration of the virus isolated from the tick of Mogould and was infected not only by mouse but also by man and was identified to be a disease identical to Q fever. After the case of Dyer, the following cases were observed:

| | | | |
|---------|------|---------------------|---------|
| Naples | 1945 | Robbins & Rustigian | 7 cases |
| Montana | 1948 | Oliphant & Parker | 1 case |

In this exposition we must also point out that in addition to the occasional visitor, there exists a third group of infection which in the literal sense could not be considered as laboratory

infections. In the question of this disease induced in two medical men who were attached to the cardiological laboratory of the Institute and of four sick persons who were taken to the electro-cardiograph department for examination, but in no case did they stay there longer than one hour. The infection was produced through the mistake of an orderly who cleaned the room with a broom and dust cloth which was supposed to be used only in the microbiology laboratory. The epidemic was stopped only when the electrocardiograph was taken to another location and the room was subjected to an energetic disinfection.

8 All these laboratory infections took place even though the most minute precautions (prophylactic) were taken. When research ticks determined experimental direction, it must be said that when the virus goes even to the embryonic egg or tick and thus come to make large amounts of cultivated virus or in the passage to the tick can develop particular properties that increase the virulence.

In respect to the pathogenesis of such laboratory infections it it can be ascribed to the inhalation phenomenon; in fact, in the Ft. Bragg Laboratory in 1945, all the experimental workers that were working with the volcanic source (strain) were infected with the exception of two, who, when working, wore masks (CARD).

Among our cases of laboratory infection there were some that were due to virus research (7 cases), some among occasional visitors (2 cases), some among frequent visitors, medical doctors (6 cases) and patients who had visited the laboratory of electrocardiography, and

all with the exception of two bacteriologists had normal disease with pseudo-influenza syndromes, with typical pulmonary infiltrate which was observed radiologically. The two bacteriologists did not have any forms of the evident disease, but in a week the virus developed in embryonic chicken egg culture and for a few days they did not feel too well, had some fever in the evening, without any other subjective disturbance or objective fact, but still could not carry on their research work. The infection was determined through the deviation of the complement which was carried through all the personnel in a systematic manner for several months during the whole period of research and eventually found that later on the two subjects obtained a constantly negative report.

The serological examination maintains itself in the majority of the cases positive for six consecutive months. Numerous radiographic controls are repeated systematically and the series have not put into evidence, with the exception of these two subjects, the presence of a pulmonary infiltrate.

With respect to the other 13 cases of infection in our laboratory, we can easily go back to the common pathogenesis of inhalation whether through the means by which the virus is stabilized or whether through the characteristics of the same disease (feverish disease, evident, typical pulmonary infiltration) in these two bacteriologists the infection was typically inapparent in development

without fever or with minimum fever, especially the absence of the pulmonary infiltrate that was characteristic of the form of the disease of inhalatory origin. We believe that in the bacteriologists the infection was derived from another means of infection and probably through the percutaneous way which have demonstrated Blank and Coll, if there is no pulmonary reaction, causing inapparent disease. The infection obtained through brushing of the virus with the tip of the fingers. In fact, at that time it was common to put the virus into the embryonic egg. It was made with out sterile cap and without sterile gloves. Thus it was possible that the skin of the hands could come into constant contact with a large concentration of the virus, facilitating virus infection.

As a second group of infection originating in the laboratories we can consider the epidemics which result following autopsies of men or animals who died of Q fever.

The first group of these is represented by an explosive epidemic which hit the personnel of the anatomy room from 14 to 38 days after the autopsies of animals or men, dead of Q fever, these times corresponding to the incubation time of (Q fever) spontaneous infection of Burnet's rickettsia.

The first episode of this kind is described by Torzani at the Anatomy Department, University of Florence, from 30 to 58 days after the autopsy of a dead patient, who was diagnosed as having pulmonite virus and the autopsy took place on 21 January 1947.

The first of the following March, five doctors and ten students that assisted as spectators to the examination without taking part directly in the operation and without having touched the cadavar or the instruments. Q fever was diagnosed in these cases only several months later after their recovery by the means of the deviation of the complement (Babuderi) instead it was not ascertained that Q fever was the cause of the cadavar, which according to anatomy had only atypical bronchial pulmonitis.

The second epidemic happened in London at the Royal Cancer Hospital following the autopsy of a cancerous old man, dead through pulmonary form which was not diagnosed as infective and according to post-mortem serological examination, it was successively ascertained as Q fever. The doctor who performed the autopsy, two assistants and an orderly were sick.

The third epidemic described by Back and Coll amounted to two orderlies in a surgical room through an analogous condition.

The fact that in these cases not only the doctor performing the autopsy and his assistants who had touched the corpse and especially the hands directly became sick, but also others(students) who had only looked on without direct contact, thus we must necessarily conclude that they certainly contacted the disease through inhalation of the virus, which leads one to believe;

(a) The virus concentration in the autopsical bodies and especially in the lungs, must really have been important (in the London case one

was concerned with acute edematous bronchial pulmonitis of Q fever origin).

(b) The quantities of inhaled virus sufficient to cause an infection must really be very small.

In what concerns inoculated and assigned personnel, one must exclude the possibility that the virus came into direct contact either with the mucous or with small skin abrasions.

A group which should be finally added to laboratory infections, consists of types of epidemics in slaughter houses, among the butchers and the clean-up personnel, after butchering animals (bovine and ovine) which, despite the fact that they had been passed as healthy by the veterinary examinations, resulted contaminated with Q fever, completely inapparent and despite this showed great infective power during a post-mortem examination of the blood and organs.

The first cases of this type have been described by Derrick, who refers to it as a butcher's occupational disease, very common in Australia and known as "slaughterer's fever".

In America, Topping and Goll speak of 40 cases noted among a slaughter house at Amarillo, Texas. Two of these cases died; however many cases classified as inapparent illness were only discovered by means of systematic serologic tests. Shepard describes 30 cases of Q fever all of which arose among the workers in the slaughtering section of a large Chicago meat packing plant; later during a systematic examination of the entire personnel in that section, 33 out of 81 men had a positive deviation of the complement, proving

to be infected in an inapparent way. Geell and Engel speak of a similar illness (20 cases with 1 fatal) in the Strasburg slaughterhouse,

In connection with these epidemics, one should note that among the best studied American cases, the men concerned with evisceration, those who are in more direct contact with the abdominal organs and with the lungs and the slaughterers who are more apt to be covered with blood, proved to be more easily infected with Q fever (Hooper, Topping and Coll).

Even though in this last group of epidemics, the relationship, that is, virus and infective relations, the simple fact that the mechanism is less simple; in addition to the inhalation way, the possibility of direct contact is excluded either through mucus membranes or a slightly abraded or open skin, contact with blood or infected material, thus these possibilities of inapparent infection, without eventual pulmonary infiltrates.

2. Another group of epidemics with sudden massive beginnings of a large number of contemporaneous patients which can be traced to direct relationship with the virus, conserved actively in the dry state. It must be said, that is, through a mechanism, a little bit more complex, that the preceeding one and which is conditioned by three necessary factors:

- a. The existence of the virus for a long time in the dry state, conserved actively in straw, sawdust or shavings.
- b. The transport of this virus to a place distant.

c. The contact of this virus with receptive persons.

A characteristic episode of this kind is then described by Vogmann at Coira in Switzerland in a mechanical office which had received machinery from America. 19 cases of operators of that shop became sick after having unpacked the machinery which was packed in wood shavings. The virus which had been conserved in the shavings and in these packings had survived the long trip.

Such a pathogenesis can also be related in epidemic in Kaifi (Wong and Cox), 200 cases and among the military of Mt. Coneri, Switzerland which were a motorized unit of artillery on maneuver (Gsell) and one of the other cases, the virus was probably conserved and transported among the straw, in the first case contained in a steamboat, in the second case used in military bivouac, in one of the other cases (both cases coming from places of endemic and of common infection). As we have seen, straw, shavings, packings, etc. can easily contain feces, eggs and remnants of infective ticks which can hide during the hibernation and which, to become infected feed on the blood of infected animals.

In other cases the epidemic assumes the character even more clearly when related to specific professions dealing with wood or raw products. In both cases, whether through the cutting of the trees or among the openings or worm-eaten holes of these, it is easy for epidemiological antropons to find refuge there, which when pulverized can take their place among the infected dust

which, dispersed in the environment during drying, can communicate the infection by inhalation.

A typical example of an epidemic through workers, working with raw materials is described by Forconi and Salvato among the carpenters and other workers constructing the electrical center in Alto Adige. Another epidemic ascribed to wood-work (Gsell) took place in Aarau, 45 workers in a wood furniture factory were in contact with dust and sawdust of infected wood.

A chronic epidemic focus was interesting which occurred in a hospital (Asylum). Among those working in the carpentry work, cases of Q fever were observed at diverse intervals over a period of three years and probably came from working through the demolition of war barracks, made of old wood, and probably were thoroughly infected with arthropods, which were probably infected.

3. Another group of epidemic episodes poses as a factor of the disease of man; the disease is unchanged when there happens a direct and immediate contact between a receptive man and an animal bearing the infection or the virus can serve actively in the dry state through the hair of the fleece, and when it is in condition to easily be fused into the environment through varying eventualities, and to give rise thus to infection of inhalation to many individuals at the same time and contemporaneously to create a true and proper epidemic explosion. A prototype of such is the epidemic of

Chiaravalle (Patrigiani and Bacchiocco) where a hundred or more persons were stricken at the time in that city and they all lived in a single street of the city, through which passed in the mornings to go to the pasture, a herd of sheep and goats which had recently arrived from Puglia for wintering and were shown later to be infected. Due to the preceding illustration one must think of the mechanism of inhalation of infective powder carried by the animals in the fleece in the dry state and thus easily volatile.

Thus many epidemics can be explained as observed by various authors in these latter times in the agricultural zones of central Italy, especially in the Marche and in Tuscany. The uniqueness of this mechanism is probably the thing to which can be attributed a true collective explosion, whether from a small single focus or eventually perhaps individual infection of a person, who was accidentally contacted, either casual or perhaps even momentaneous, with an animal infected and bearing the virus, perhaps by contact through the hair or coat, poorly cleaned, dusty and infected with ectoparasitic arthropods or some of their remains. This mechanism of infection can be created in addition to the animals of which typical examples are the sheep, with abundant coats, but also the equines and the dogs, that which, without having necessity to touch or reach them, a current of air can bring about gusts of infecting powder.

4. A large group of epidemics with a mechanism even much more complex is related by the epidemics which are obtained in the city and the agglomerations which are found in regions like Sicily where there exists an endemic and zoonotic Q fever in the countryside.

These particular epidemic episodes present a particularly exceptional character that we have not been able entirely to hit upon in Sicily in our systematic study of the disease. These epidemics of the city or human agglomerations have always gone along in a markedly stage march, that is, in various stages; they are more frequent in the period which exists between the end of autumn and the beginning of spring, although its appearance at other times is exceptional but cannot be completely excluded. In general, the epidemic does not come by itself but usually is accompanied and sometimes mixed with influenza or pseudo-influenza waves, with which the cases of Q fever are mixed so that it is not possible to calculate the exact proportions in view of the patients stricken with influenza. It is not possible at the present state of conditions to determine, except only purely approximately, the numerical amounts of the three epidemics of Q fever which were observed under our direct control in the city of Palermo in 1948. In general, the patients did not show up as groups in a determinate region or groups of houses or even in a single family, it was, instead, an epidemic of various cases which were distributed throughout the city in different parts of the city but which occurred at the same time.

We cannot say that there are certain wards or sections of the city (for example, in the vicinity of the harbor, railroad yards or in the periphery next to the countryside) which in those years had larger frequency of Q fever cases, or that even show the particular tendency to those. Only rarely did there occur as much as two simultaneous cases in a single family except perhaps sometimes in the cases of pulmonary form, the sputum of which we know is highly infectious. Sometimes with a certain amount of frequency also in the city, there can occur sudden cases in a house which occur at different stages during and in the course of different epidemics so that the distance of time between the appearance of these single cases makes it possible to exclude decisively any possibility of any direct contact between patients or of a unique inflicted moment which could have resulted in these cases, if not at a contemporaneous time, at least, a relatively neighboring period of time.

Many of these characteristics of insurrgents of these epidemic cases in the city did not seem to be analogous to those which characterized rural endemics.

From this data it would seem possible to put this group of epidemics, characteristic of the city in the agglomerations of Sicily, in single intermediate position between the epidemic form and the endemic form.

In effect, following our study, it was possible to demonstrate

that in Sicily the epidemic episode happening in the city and in the large rural agglomerations were demonstrated constantly to be in pathogenic report or accord with the rural endemic which was always produced through diverse mechanisms which are:

1. Through the transport, by means of hairy animals, different dusty objects and filth (straw, rags, etc.), work instruments, vehicles or wood with animal traction, etc. - of the virus in the dry state, conserving its powder easily to lift up into the air and to be disbursed, determining the possibility of an infection through the inhalatory means; or

2. Through the milk of infected animals, or aliments dirtied with excrements or whatever other infected materials which can create ingested infections which can represent a possible mechanism that could be determined.

In this respect it is of interest to note in particular of no small epidemiological importance of the cities of Sicily, Palermo included, and for much of the south; it is that in the morning many groups of milking animals (milk cows and goats) come in groups from the country to be fed directly in front of the houses and that a systematic examination conducted by Mirri in Palermo showed that almost 50% of them were infected with an inapparent form of Q fever. It should be observed that the housewives having personally observed that the milk was taken directly from animals apparently in good health, commenced to put it to boiling and thus

eliminated the possibility of contagion, either by infection by those who drink it or by inhalation by those who handle it. These intermediate situations appear even more exceptional when it is considered that the determination in these groups of an epidemic factors of climate, location, etc. opposite to the other types of epidemics has such an importance that they dominate through unrecognized mechanisms the whole epidemic march.

These facts are even more unusual or strange in respect to:

1. The transport of the virus from the infected countryside to the city can occur at any moment through any filth or dust carried by the workers who come into the city, or hairy animals that for different reasons can come in, and finally through the milk or other infected foods; in the infected animals which are in the continual conditions of eliminating rickettsia through the milk (Jellison) and the urine (Moldolesi, Weismann), etc. and thus to create infections from which derives the fact that cases of endemic Q fever in the country can be observed in every season.

The fact that epidemic factors become effective only in particular conditions of season and climate makes us necessarily think too of the intervention of factors related to the life and the development of ectoparasitic arthropods since, as it is known, their importance as direct vectors of the disease, at least in America and in Europe, must not be too much of value. In the cases of the Sicilian endemic and epidemics since there was a positive

report of a massive infection of animals at the same time as the epidemics, those occurring prevalently in the cold season while the infection of animals through means of ectoparasitic arthropods and especially of ticks is always major in the cold season. On the other hand, our knowledge of the virus in ticks assures that an animal surely infected although this information comes from non-systematic investigations of small number, are sufficient to exclude the fact that the tick in general can globally be infected in such a percentage that it would institute a permanent reservoir of the virus from which the epidemic could come.

But we have not demonstrated that for an infection for Q fever in which the inhalatory means represents the most common mechanism for infection for man that the tick possesses importance as vector of the virus, in maintaining and stabilizing the infection of the wood dust or sawdust of the shavings, etc. and intervenes in the mechanism of individual infections of epidemics, etc.

From all this it can be derived that the seasonal cycle of development and life of the tick, also through the emission of the eggs infected through heredity can come in some of the means from which is due the seasonal veribility of the urban epidemics. In fact, the degree of infection of the dust capable of creating of inhalation infections, so important in the epidemics, depends in a major or minor characteristic of the infected remains of dead ticks or their excrements and these are major or minor following the seasons and with relation to the season and the climate, exercised

unto their development and the fecundity of the ticks. Since the ticks in general at the approach of winter hide in the straw, the dry wood or the barn dust and go into dormancy, it is easy to explain why the dust and powders are more infected in the cold season and that infection through inhalation of carried virus is easier during the cold season and this had been verified in the Sicilian cities.

It is also possible that, as it has been seen, the coefficients with respect to the seasonal evolution can come in, multiplying the conservation ability of the virus in the powders or dust and consequently their infecting capacity; these physical coefficients could be, for example, dry climate joined with more active conservation of the virus in specific seasons and decreased the activity with humidity.

Clinical.

The clinical aspects of Q fever are much more varied. These after all have passed the initial concepts of an acute form and of pseudo influenza frame, with a limited possibility of symptomological variations and being, in a quantitative sense, through the various gravity and intensity of the attacks; and with very little amount of variability of the symptoms in the qualitative sense, determined only by the supposition of the complications which are very rare. Actually, especially through the contributions brought by the European observers, among which the observations of my school are of no little

importance, the clinical frame of this disease appears to be very varied, frequently in other than the acute form, sometimes in the subacute form, the one and the other with the possibility of focusing into clinical syndromes of diverse aspect and nature, which reach to their origins in anatomical lesions and functions of different organs or apparatuses and extending their symptomology to those of pathology from which they are not too far.

I have the clear conviction that in spite of the new and important knowledge derived from clinical and experimental contributions of these latter days, the edifice of our knowledge on Q fever cannot be considered at the actual moment still complete; and that any clinical contributions must be considered still truly important for it is exactly guaranteed that they can still inform us in regard to the pathogenesis, not only of the entire disease of the subject under discussion, but also of the particular disturbances and particular lesions that the patient possesses. In fact, it must always be maintained in mind in this particular kind of research that once it has been established with strong certainty that the patients under examination infected with a form of Burnet's rickettsia (active) it is not questioned for this reason to automatically authorize, to attribute to this infection any existing lesions in the disease since, in the majority of the cases of acute infection, it has always presented a possibility that other lesions can occur in an affected subject, lesions which may be chronic or subacute, pre-

existing of inactive or latent state; lesions which, when discrimination is applied may be erroneously attributed to new alterations of this infection, preceeding or concurrent with it, which are completely independent from it.

The diverse clinical forms of Q fever, with its many forms of symptomology, constantly presents some common and fundamental symptoms which it should always be the practice to look for at the moment of the suspecting and the establishing of the diagnosis, which are;

1. Splenomegaly - At the beginning and under the acute form, typically soft; with a prolongation of the form, if less evident, while there is a tendency to an increase with respect to consistency; in the recurring forms (pulmonary, pseudo-Brucilla) there is a reoccurrence of the fever and of the fatty bodies and a reaccentuation of the splenomegaly. The splenomegaly is a fundamental symptom and constant in all the forms of Burnet rickettsia, whether spontaneous or experimental, as well in man as in the animal.

2. Fever - It has a somewhat more varied aspect until the beginnings of the disease; a high fever to a moderate fever or the small fever; it can be continuous, intermittent continuous, relative continuous, irregular; often it has a clear undulating characteristic and is accompanied by profuse sweating and general disturbances (malaise, headaches, anorexia, nausea) which are reaccentuated each time.

The duration of the febrile period can be quite varied, from a few weeks to several months.

3. Pulmonary infiltrates - With the exception of the cases of smaller or low fever, of which we shall speak later, it is always first with major or minor evidences from the beginnings of the disease; but it can vary quite a lot as to its duration, its intensity and its localization from case to case (Longhope). In the more common cases it has typical character of a transient infiltrate. Pathologically, anatomically, speaking it is made up of concord foci of broncho-pulmonitis with a kind of iperemia extending to the bronchia and with intravalvular exudations. In the majority of the cases it has a tendency to regress, sometimes up to a complete reabsorption. In the most acute forms with a bronchial pneumonic character, the infiltrate, once localized in a determined portion of the lung, as observed diffused with small sparse nodules and also numbering in the thousands. In these cases it is shown to be surrounded by large and characteristic halo of inflammatory edema. In the subacute forms it can be accompanied by peribronchial, monocytarian infiltrations or perivascular infiltrations of connective reaction, where a major persistence of the infiltrate exists, which comes to lose its characteristic of transient infiltration.

In the acute forms the transient infiltrate cannot give a symptomology to common or simple examination of the chest, since the infiltrates exceptionally are superficial and can be diagnosed in a direct clinical examination. It is easy, however, to demonstrate it through a radiological examination where this kind of transient

infiltrate appears as a tenuous shadow, undefined, with a traumatic structure, especially located in the pulmonary spaces but sometimes visible in the higher zone, non-excluding the pottoclavare. This shadow of infiltrate persists differently from case to case, in the more common cases, after one to two weeks it can be considered as a relic.

It is important to point out that there does not exist any parallelism either in intensity or duration between the infiltrate and the fever.

Many infiltrates can be accompanied by an average or moderate fever, while in patients with a high fever, it is possible to have a tenuous infiltrate, small extension of very, very little symptomology, even though radiologically significant.

And it can also be that even though there exists no traces of the infiltrate, a small fever can persist for months.

In the recurring pulmonary forms, the infiltrate remains unchanged for some intervals of time when there is a general disturbance and finally coughing and the expectorate disappear; the total reabsorption occurs only and solely when the recovery is definitive.

And in general the fever disappears rapidly with aureomycin (Lonneste, Lonneste and Coll, Rosove and Coll, Wong and Cox); sometimes the infiltrate or complete stoppage of the incubative process can disappear after a few weeks or in some instances in two months.

4. Negativity of the normal laboratory examinations - normal urine. Blood without any particular morphological characteristics, quantitative or qualitative, especially of the leukocytes. From case to case independently can be observed that a moderate leukopenia or a moderate leukocytosis; moderate neutrophilia or a relatively evident lymphocytosis. We cannot confirm the affirmation of Gould that the active form has as a characteristic an eosinophil; in many cases however, we have observed especially at the beginning a tendency for small eosinophilia (Ingrassia).

5. Sedimentation velocity of the "emazie"; it is constantly increased, in the repeating cases when there is a new attack it is preceded and accompanied with an increase in the sedimentation rate.

6. Hemagglutination in the cold - in Q fever on the contrary to what it is in virus pulmonitis, it is constantly negative; this symptom has a differential diagnosis value. The observations of Heilmeyer of cases of positive cold hemagglutination have not been confirmed.

7. Arterial hypertension, venous hypertension, marked "asthenia" - persists even in convalescence.

8. Behavior of the electrocardiogram; Brawley and Modern speak in a case of six examinations of electrocardiographic alteration (deviation of the electrical axis to the right). DeOrchi has followed systematically 22 cases of electrocardiographic behavior during the disease and in convalescence, in 13 cases, the graph showed no path-

ological alteration; these were light forms of moderate cases with a transient infiltrate, rapidly recovered, either spontaneously or with aureomycin. In 9 cases, however, there were evident alterations present of a myocardiac-coronary character. Of these cases, 4 showed serious alterations of the T-wave in the third Der. and in the precordium with marked levelling of the S-T graph. In a young woman with a pulmonary form of moderate proportion, the electrocardiograph alterations were evident for several months after the clinical recovery of the patient. In a case of special gravity with an edematose bronchial pulmonitis and with circulatory collapse after the fever, the electrocardiograph examination showed persistent alterations of the myocardia type, these alterations remained evident for several months. In general the alterations of the electrocardiogram observed during the fever disease have been demonstrated to be modified by aureomycin therapy.

9. Completely negative results of the nemo culture on common medium and complete negative results of the common serological investigation, reaction of the wild phalex pad. The negative results of the wild phalex reaction can have a discriminatory value when confronted to other rickettsia for an eventual differential diagnosis.

With respect to the behavior of the reaction for plague and especially of the Wasserman reaction, only DiCastro has not observed a positive result for Q fever. We have in 12 cases had positive results at low level, limited however, during the duration of the

feverish period when the fever abates the positive result is scarce.

It seems to us that this problem is worth studying systematically in other cases of Q fever: in other cases of a typical pulmonitis with positive Wasserman reaction, the eventual positivity of the serological reaction for Q fever must be studied systematically for typing, it in with the biological proof in guinea pigs in cases where this would be positive. In such a way it would be possible to correlate the reports for Q fever and virus pulmonitis described by Hoglund, Grumba and DiFacconi.

In the clinical study of the cases of Q fever which have been reported in the world literature and including the contributions of our personal files, it is possible to distinguish among the many forms which call be related to the constant presence of some common symptoms that we have considered as fundamental and as disease.

These different clinical forms are :

1. Pseudo-influenza form.
2. A settic form.
3. A broncho-pulmonitic form.
4. A nervous form.
5. A pseudo-brucillus form.
6. A form of small fever (Burnet's little fever)
7. A sub-acute pulmonary infiltrate.
8. A form of latent disease with apparent symptomology.

1. The pseudo-influenza form was first described by Derrick. It occurs easily in the cases reported by American and Australian authors, frequently it is presented in epidemic episodes and especially in those where there is particular gravity of the situation. The general aspect of this form falls very well within the framework of a common attack of influenza and is differentiated from it only through the duration of it, which is always major or larger.

This form has always an acute beginning, at the time of good feeling and without any previous period of attack; it is accompanied by shivering, of a larger or smaller intensity, which can be more or less generalized, there develops malaise, debility and of general depression. There is also myalgia and diffused arthritis of the rheumatic type, sometimes there are chest pains (Robbins and Regan) which provoke exacerbating respiration pains of the pleuritic type (Finestone and Coll). In addition to the fever in some epidemics there are some kinds of catarrhal, more or less intensive in the pharynx (Robbins and Regan, Finestone and Coll, Meldolesi) and of the higher respiratory tracts. Some of these lack in other epidemic episodes; not infrequently there is a persistent cough, dry or with little expectorate, sometimes mixed with blood. Not too rarely the beginning is underlined with evident hemorrhagic manifestations; these can be more or less intense or of small importance (Irons and Hopper, Meldolesi).

The general appearance of the patient is very similar to that of one suffering from influenza, congested face, red conjunctivae. In the first two to three days there can be, not infrequently, a cutaneous rash on the face, breast, shoulder, back (Raleigh and Modern, Derrick): almost always there is a feeling of mental and sequelous confusion, especially on the bronchial locations, also in the retroorbital location; in any case there is observed a pain upon movement of the ocular bulb, typical of influenza. There can also be a state of disquietude and insomnia, but it can be observed not infrequently a true and proper stupor state or a state of mental confusion and also of intense agitation (Derrick); in some cases it can also be added a generic sign of menengitic suffering; photophobia (Derrick) and nuchoridity (Honnebruck and Nelson, Robbins and Ding). The tongue is always diffusively patenate, there exists sometimes digestive disturbances more or less marked; anorexia, nausea even sometimes vomit; also sometimes diffuse pains in the abdomen. Many times there is evidence of diarrhea (Robbins-Megan, Brawley and Modern) but rarely is there a marked constipation. Especially there exists a burning urine, a symptom that the Anglo-Saxon authors have underlined with insistence. In any case, Raleigh and Modern have encountered a micropoliadenia which is very painful and diffused to the major part of the lymphatic glands. The objective examination is completed with this in addition to the splenomegaly and the pulmonary

infiltrate which is more or less marked and more or less persistent from case to case.

The fever is always rather high and continuous, it is remittently accompanied by profuse sweating. The duration of the period of fever varies from case to case; from 10-20 days, from rapid exhaustion to complete recovery, without any type of relapse, excepting a profound asthenia which lasts for a few weeks.

2. The settic form is characterized by generic impression of great seriousness which emanates in type from all the symptomatology, completely pervaded by the profound and serious general setticemical character; definitely settic tongue; evident and remarkable depression of the general condition, of the sensorium, of the circulatory functions; high continuous or intermittent fever; constant myocardial coronary changes which can be shown electrocardiographically (DeOrchi), marked arterial hypotension, venous hypertension. The duration will be at least from 4-5 weeks. Complications can set in very easily, the most frequent and the most characteristic among these is broncho-pneumonia of a migrant nature, with reactive pleuritis (Maldolesi, Magrassi and Coll, Coppe and Coll, Irons and Hooper, Noesthlin and Koszowski), and above all phlebitis, (Huebner and Sticknel, Magrassi and Coll, Coppe and Coll, Maldolesi) pericarditis (Maldolesi), orchitis (Irons and Hooper) and also Maldolesi; Csell; Noesthlin and Koszowski; angitis, (Maldolesi)

pancreatitis (Hoeschlin and Koszewski). The convalescence under these forms is very slow; functional reconstruction is slow. A certain percentage of deaths exists.

3. The broncho-pulmonary (broncho-pulmonary edematous) form represents a characteristic of Burnet's rickettsia, either the clinical study or anatomopathological discovery.

It has always a rapid beginning, resembling true pneumonia; in full health with no premonitory disturbances, with sudden shivering and immediate state of exceptional gravity and of profound depression of the general state and of the circulatory function; high fever, continuous and noticeable arterial hypotension. Low, frequent pulse, which is thick, filiform, rapid; dyspnea and violent cyanosis; persistent and tormenting coughs, with serious expectoration of pink coloring, in every way like the characteristic type of acute pulmonary edema. During an examination of the thorax, a reduced tympanic sound came to light, spread through the entire pulmonary region. With great attention during the examination, at a certain point here and there one is able to detect the vaguely traceable sounds of bronchial pulmonary confluence. However, the vascular murmur completely vanished and was replaced by fine regular râles; breathing similar in all ways to that of acute pulmonary edema. This simiological report true to characteristic, finds a perfect interpretation in anatomal-pathological report (Lillie and Coll; Harman; Maldolesi) which brings into evidence spread here

and there, multiple foci of broncho-pneumonitis, of confluent character, enclosed by a large halo of highly contagious edema.

Six such cases have been brought to our attention, which can be compared to the case of contagion from a laboratory described by Lillie and Coll and to Harman's cases. Cases of extreme seriousness are always discussed, which can easily result in death, as in the case of Lillie, in that of Harman and two of ours.

Such a case in our experience demonstrated an even greater and more precipitous clinical syndrome without referring to particulars of the individual. It concerned a young artillery officer, 26 years old, no particularly weak background traits; he was not plagued and not a smoker; strong with no original deficiencies, with no previous weaknesses of any kind. Attacked by the illness in full health, his friends and doctor were impressed from the very start by the sudden state of depression and of seriousness. By the third day of sickness, conditions were suddenly precipitated to the point of a state of collapse, protracted 48 hours, with a hippocratic face, very dangerous arterial hypotension; the pulse hardly noticeable, rapid, irregular and arrhythmical but above all with complete suspension of the diuresis and a very dangerous state of secondary hyperazotemia. He slowly recovered with symptomatic treatment. The electrocardiographic examination showed grave myocardiocoronary changes of the myocardial types (DeOrchi).

In certain cases, especially if one uses aureomycin, the fever begins to drop rather rapidly, after 2 to 3 weeks, leaving the patient in such worn out condition, from a general point of view and because of circumstance as to necessitate a few months of convalescence and a series of symptomatic treatments before there can be a sign of recovery.

However, in other not rare cases, a typical symptomatology of successive relapses is observed, each lasting from 3 to 4 weeks, recurring at intervals of varied length of one or more weeks during which the patient, while enjoying a relative lull in the symptoms of the illness, mostly the burden of the respiratory system (coughs, dyspnea, expectorations) does not indicate any lasting improvement. Then, at every relapse, the high fever, the sudden gravity, the heart and breathing difficulties and the burden of the respiratory system reappear suddenly.

To the characteristic clinical and anatomo-pathological discovery concerning the lung of this form correspond, a radiological discovery even more characteristic, i.e., a shadow, not uniform, in the two pulmonary cavities, more marked at the bases, so as to nearly always leave the top sections unaffected. This irregular diminishing of transparency of the pulmonary areas is due to two different conditions:

a) to a circulatory effect, of infectious pulmonary edema, which is the more striking and more evident, depending on the acuteness of the type, whether at the start of the disease or of every eventual successive relapse.

b) to the combinations of very numerous dying particles, roundish, of the sublobular or lobular type and sometimes also sub-military which are especially more noticeable in the high regions of the pulmonary areas where they always join smaller, and therefore, more isolated; while in the lower regions where the tendency is to combine, they do not appear unless from the extended shadows, irregular in distance and transparency. The more the illness is separated from its beginning, the less diffused appears the dedmatous area, while on the contrary, the shadows of infiltrative effects, sub-military, become more and more dense and with more and more blurred contours. In a successive period the character of the micronodular diffusion continues gradually to disappear because of the strengthening of the phenomenon of confluence.

The type, which is typified by relapses, presents noteworthy modifications from one period to the next. During the intervals, the edema fades, however despite the stopping of the dyspnea, from the cough, expectoration, the nodular shadows not only do not vanish, but they have a tendency to become more intense and larger, with ever more precise contours, while at the same time a conjunctive reaction of the peribronchial and perivascular character appears in the background

which progressively grows in intensity and magnitude. During each new relapse, the edema halo reappears, with the reappearance of acute clinical symptomatology on the basis of this radiological background. However, the edemic phenomenon shows a constantly lessened intensity after each new relapse, while, even their sub-nodular shadows, recent and with blurred intervals joins the existing ones. Only at the final healing of the disease do these infiltrates resolve, more or less fast, while the peribronchial reaction remains repeated at length.

In these cases of edematous broncho pneumonia we have made a singular and characteristic discovery. If, at the beginning of a relapse or during the acute phase of the illness, we injected the sputum of the patient using the necessary technical precautions, into the peritonium of a guinea pig, we could establish a typical Burnet's disease(in the animal), which could easily be transferred into the animals in series and even into the embryonic chicken egg until the pure culture has been obtained. Already after the first attempt, in the guinea pig's spleen, which has reached far larger proportions than the normal biological reactions, coloring bands of pulp using Macchiavello's stain or better, using that of Giemsa, the undisputable presence of tickettsia. I repeat, that through our personal experience, it is not an ordinary happening during biological problems in which common experimental materials are used.

Thus it is proven that among patients of this singularly particular type, a direct elimination of rickettsia takes place through sputum. The amount of virus in the sputum of these patients must be important, for, very frequently, the guinea pig, 5 days after first injection, demonstrating the characteristic of the report of rickettsia Burnetii. A reaction observed by the common biological experiments of rickettsia in the guinea pig for this, in type, is quite easily tolerated by the guinea pig, not infrequently, with the passing of time, hardly any signs of illness. Another effect, singularly important, is some observation on these patients. In one of our patients, in a clinical record of particular danger and gravity, at the beginning of the disease, the serum which contemporaneously to the biological experiment has shown that it contained a large amount of virus infected the guinea pig, examined on an ordinary swab colored with the method of Macchiavello or of Giemsa, showed a great quantity of minute bacillos, which could be identified as rickettsia. Their shape, size, a unique report in science today (Duttu).

4. Nervous troubles are not very frequent. It was first observed by a patient with a particular clinical syndrome. Symptoms of nervous changes, central and peripheral, during Q fever have been recorded by the author in cases of epidemic episodes of

NOT REPRODUCIBLE

lesser gravity; headache, vertigo, a feeling of general malaise, profound asthenia (Hornibrook and Nelson), intense and persistent headache (Derrick; Roblins and Ragan; Card; Hornibrook and Nelson; Shepard; Brawley and Modern) which frequently constitutes the most obvious symptom during the entire most acute part of the illness and can also persist for a long while during the convalescence, after complete lapse of fever (Meldolesi). In acute cases, more or less important changes affect the sensorium (twisting, a stupefaction); Derrick speaks at length of the possibility of a true and actual typhoid state, accompanied in many cases by photophobia and by rigidity of the neck (Brawley and Modern). However, we have always discussed highly fevered patients, acute, of particular gravity, in which the nervous state can only represent the expression of the general toxic state or yet, of the acuteness of the danger of the disease.

While the nervous state described by us always has a rather less acute character and manifestations, it nearly always manifests itself with low fever, in certain cases with only a little fever; however, in every case the fever sequence appears in waves of varying duration, recurring at intervals of various length, during disorders, always of organic nature, present ulterior rekindlings. Characteristic of these nervous manifestations for the entire duration of the illness is the constant presence and persistence

of splenomegaly or of pulmonary infiltrate. The disturbances, in the form of lesions of encephalitic character, always begin in a sudden manner, without ever being accompanied by attacks, even minute, of the sensorium, the sleep and of the oculomotorius synergy.

A characteristic form, noted by us frequently, is represented by the grouping of paralysis in the areas of the pharynx, the larynx and of the velopendula with dysphonia, dysphagia, dyspnea; frequently unilateral but bilateral in some cases. The appearance of an associated syndrome of this type, completely isolated, during the course of an illness with hardly any fever, without any disturbances of the sensorium must make us think of Q fever. In one such case observed by us, the motor difficulty in the area of the larynx, which started suddenly, was so serious as to cause a paralysis in the area of the vocal cords and to force an emergency tracheotomy, to avoid serious asphyctic possibilities.

Usually, the disturbances of impulses, especially under treatment with aureomycin and vitamin E, but sometimes also spontaneously, can regress slowly and without after effects. Wegmann, Moeschlin and Koszewski describe a typical encephalitic extrapyramidal syndrome of Q fever, with dylopia, reversal of sleep, speech and sensibility disturbances with slight motorial deficit of the pyramidal type, this syndrome too can regress completely. Another not infrequent, form manifests itself by diffused pains, of polyneuritic type, frequently accompanied by a certain sign of meningitic

suffering. The pains sharpen every evening, with the rise of temperature, which remains low, they also reappear with each new fever period, during the not infrequent relapses. By type, these pains remain for a very long time and decline very slowly.

Another form is characterised by headaches, which can appear:

a) either as an attribute of a common acute attack, pseudo-influenza and later even after this is exhausted, continues as a low fever, persistent for weeks or months.

b) or as a phenomenon alone and isolated, only accompanied by enlargement of the spleen, by pneumonia infiltrates, by waves of fever, after assuming a violent tormentous character, especially during the heightening of the fever, which are also noted by the transitory appearance and flight type of meningeal irritations (photophobia, light rigidity of the neck, trifling hypertension state of the spinal fluid).

In both of these forms, the headache is always persistent and slow to regress. We have noticed it sometimes continues slightly modified, a long time after the disappearance of fever and of the pulmonary infiltrate, when one could even have stated that the clinical healing had been completed. Moeschlin and Koszewski describe during a case of Q fever, a typical form of acute lymphocytic meningitis, with a rise in the spinal fluid of both the cellular elements (58) and the proteins (125mg%); Roch and Coll, and Mach

have noticed as such.

In all neuritic forms, whether observed by us or others, the glycerol has never shown significant changes which could have a differential value. We have never observed the presence of antibodies revealable by complement fixation with following Robbins and Ragan, a positive biological reaction when injected into a guinea pig.

5. The pseudo-brucella form is not infrequent. It follows the clinical pattern of brucellosis completely, accompanied by typical fever waves, profuse sweating, prolonged course, through successive waves for months and months. A differentiation between the two forms is only possible through diligent, precise and repeated laboratory serologic and microbiologic research.

6. The fever form is also not too frequent. Here it is isolated among the speckled groups of patients who present themselves to the doctor for the solution of the complex and difficult problem of long lasting fevers, resistant to a great variety of treatment and not resolved through the usual and more exact clinical and laboratory examinations.

The Burnet fever, usually lasts for months and months without greatly upsetting either the general state or that of nutrition or blood count; it is never accompanied by particular subjective or objective disturbances with the exception of some shivers with the rise of temperature and slight sweats with fever decrease and by

some general disturbances, of mostly cenesthopathic character. The objective report, if one excerpts the enlargement of the spleen, which is often also negligible, is practically negative. No trace of the pulmonary infiltrate is even found during the radiological examination even if the latter is conducted at the beginning of the illness.

The existence of a Burneti fever, without appreciable pulmonary localization, even at the beginning of the illness, has found the most luminous confirmation in an endemic focus of Q fever recently identified by Pattavina and Dattitta in a penal camp (Augusta); of the consistence of 52 cases, all positively proven, at high titer, as well by the seroagglutination as by the complement fixation. The biologic experiment was also possible in 6 cases, positive reaction from the patient's blood; all had a persistent fever for more than a month, with slight splenomegaly with no subjective or objective disturbances and mainly with no signs of pulmonaty infiltrates. Even in the 6 cases, while still at the beginning and with the biological proof from the blood still recent, the radiological examination for pulmonary infiltrate appears negative.

The major part of these patients presented, during the illness, a singular exanthema, from bladder elements, on a normal skin, spread here and there especially on the trunk, which greatly resemble the rickettsia pox. We should remind ourselves that elements

similar to those noticed in the Augusta epidemic have been observed by us in other cases and by Patrignani and Bacchiocco in an epidemic in the Murales.

At the Augusta epidemic, fever soon gave way under aureomycin treatment.

7. The subacute pulmonary infiltrate is not even very infrequent. It has as an anatomic-pathological basis a mononuclear infiltration of the blood vessels and of the bronchi, with a secondary connectival reaction. Radiologically the infiltrate is more dense than in the acute form; more consistent and with well defined limits. It can be localized either parenchymal or in lymphatics; in which case it is accompanied by secondary atelectasis, caused by the compression of the bronchial tubes by the force of infiltrated glands and by remembering the plastic or tumor forms. Its persistence and its radiologic characters can embarrass in the confusion of specific and neo-plastic shapes.

a) a peribronchial type, with irregular regional alterations of the pulmonary design; with typical images of bronchi with inspected walls, either taken in a file (annular shadows, with central transparent light) or in a manner perpendicular to the section of the rays (rotating images). Such images, with linear diffusion are similar to those encountered in peribronchitic changes of various types, and are usually localized in the two outer thirds of the corresponding pulmonary section.

b) a circumscribed alveolar type; characterized by the presence in the pulmonary area, of a round shadow, compactly blurred, with irregular outlines, of slight and ununiform density. The seat of this shadow is frequently local, but it sometimes can also present itself high and even in the area known to radiologists as "subclavicular".

c) an ilo-pneumonic type, sometimes bilateral, but also frequently monolateral, characterized by massive hilous images, with direct digitation towards the various regions of the relative pulmonary area, along the vasculo-bronchial filets. They may vary in density and consistence also appearing at more or less defined intervals, so as to simulate with a narrow radiologic point of view, the images of processes either neoplastic or specific infiltrates and with great difficulty to establish a differential diagnosis.

From a strictly clinical point of view, the sub-acute infiltrate of the lung always represents a more or less intensely feverish illness, of a progressively evolving character, even though slow; after a few months it can also be accompanied by malnutrition and anemia, at the expense of the general condition; it may appear with breathing difficulties, with insistent cough, with more or less abundant expectoration, also sometimes of a definitely hemorrhagic character. Even if we wanted to evaluate the efficacy of the aureomycin cure, so as to obtain a criteria ex adjuvantibus, even at the first treatment a decline in the fever can be observed, the infiltrate frequently remains completely unmodified. Sometimes it is necessary to insist

on 2 to 3 successive cycles of the antibiotic so as to establish a safe beginning of regression and later after a few months, the complete clearing of the latter. Reference is made to cases of hardest and riskiest interpretation.

8. The form of latent illness, with unapparent symptomatology (sub-clinical Q fever) is discussed today by all world literature (Bock and Coll) and its existence is also confirmed by our case history. We are concerned with individuals who, in cases of epidemiological research, show strong enough proof of positive serologic (agglutination, fixation of complement) reactions of rickettsia Burneti, without ever having suffered in recent months disturbances or symptoms pointing to any illness, either as such or as a possibility, a typical case, in our records, is that of a stableboy working in a stable with animals proven to be infected and whose wife washed his work clothes, contracting a disease of a typical form of Q fever, also proven at the biologic examination. This stableboy, in excellent health for his years, showed a highly positive reaction by the agglutination and fixation of complement in the serum; the biological examination was negative. He showed signs of having had the illness recently, while it was not possible to demonstrate that it was still active considering the negative biological test.

An even more experimental case stands out among our case histories. Our bacteriologist, dedicated to research in the field

of rickettsia, are always kept under clinical observation. They are subject to serologic examination every month (agglutination, fixation of complement) during the beginning in 1949 having succeeded through some of our problems to pass the organism from the guinea pig to cultures in eggs; after approximately a month, two of our bacteriologists, suddenly presented positive serologic reactions, of a rapidly and progressively growing nature. Having been subjected to radiographic examination of the thorax, no signs or traces of pneumonary infiltrates existed in either of these; however, the spleen seemed slightly enlarged in volume and of soft consistency. One of the two bacteriologists had a light daily fever in the evening, not accompanied by disturbances of any weight. However, the other, despite the most accurate measuring, showed no fever changes at any time of the day, had no disturbances or ill feeling of any type and felt in perfect condition to continue work and research. In both cases the positiveness of the biologic test reached by injecting the subject's blood into a guinea pig, gave proof of an active infection, which in the first case caused a typical febrile form, with the characteristic absence of the pneumonary filtrate; in the second case it produced a typical form of subclinical, asymptomatic, inapparent Q fever.

From the epidemiological point of view, research in sub-clinical cases, both active and anamnestic, holds the greatest value in closing the link of infection from animal to man.

We hold that in the pathogenic determination of the symptomatologic multiformity of Q fever besides the common individual factors and other factors; circumstantial, professional, etc., great importance is assumed by the variety of means of infection.

From the experimental reports, we know that, for example, the percutaneous or transeutaneous infection, which can also be considered that caused by hematophagous arthropods, determines as the greatest lesion that which can remind in some cases, from a morphologic point of view, the black spot of fièvre boutonneuse. As general reaction of this type of inoculation, the splenomegaly is moderate. The fever is very low and in many cases can be completely nonexistent, the pulmonary infiltrate is absolutely missing; while on the other hand the immune reaction, shown by the positivity of the sero reactions, is always very strong.

On the other hand, the contagion, through inhalation, especially from infected dust, always causes an intense fever and above all the formation of a pulmonary infiltrate.

Among our bacteriologists, who worked under sterilized glass caps, which eliminated nearly all possibility of contagion by inhalation and managed large quantities of virus, it is not improbable that the infection took place transeutaneously, though only through the intact skin of the hands and especially through the fingertips. The mild clinical form which appeared in them fit such a pathogenesis perfectly. Thus, in the endemic focus of the Augusta penal camp, the most accurate epidemiological data were

useless in establishing the most common causes of the epidemic; nearly all possibility of infection from animals and from their products was excluded; by breathing of infected dust, etc. Given the particular atmosphere of a penal camp, the hypothesis of an infection by way of bites from ectoparasites remains most probable, also in view of the singular cutaneous vascular eruption.

The great frequency, among cases of Q fever, of types with presence of infiltrate and with the character of an acute illness with more or less high fever corresponds in this way with the great prevalence, in the pathology of Q fever, of contagion through breathing.

During the clinico-epidemiologic study of Q fever, the problem of eventual reports of clinical forms presented by the patient and the eventual means of contagion represent also from a practical point of view, one of the most important and the most interesting areas of study.

Serological and Microbiological Facts

The former are possible by seragglutination and fixation of complement; the second by means of biological experiment in the guinea pig. Murr's ophthalmo-reaction can finally be used in animals.

Seragglutination was the first method used, in order of time, to study rickettsia Burneti; Freeman and Burnet used this to reach the identification of rickettsia Burneti. However, this method, as

opposed to complement fixation, has lost favor in practice, both medical and epidemiological, it causes some practical difficulties for those who try to execute it, especially in reading the results. In 1944, however, Giroud propounded a new technique of micro-agglutination, to an extreme clarity of reading and above all requires minute quantity of antigen, which is not without its practical advantage.

We have used Giroud's micro-agglutination for experiments, paralleled with the fixation of complement, using the specific technique proposed by Buttitta for the preparation of the antigen. By which in the guinea pig, the comparison of the agglutinated antibodies makes somewhat more slowly the comparison of fixed antibodies, also, in practical results, sero agglutination has given us results in complete agreement with those obtained from fixation of complement, therefore, we are discussing a method of great practical value and one not to be forgotten, especially when one has to examine small quantities of serum at a time.

The fixation of the complement, because of the facility of execution today has become the method of choice and nearly of habit in the serologic determination of Q fever. All authors agree in their preference of the reading "cold" technique described by Jacobsohn and today misnamed the Kolmer technique by Anglo-Saxon authors.

All authors have used this method in diagnosis, both clinical and epidemiological, not only Smadel and Coll; Bengtson. This method of research contains inassailable qualities of precision and attention (Irons and Coll).

Like Smadel and Coll, we in our case histories, and especially in dubious and difficult cases, have systematically controlled the results of agglutination and of fixation of complement with those of the biological experiment in the guinea pig. In no case were any differences found between the sero agglutinations and fixation of complement and the results of the biological experiment.

As to precocity, the appearances of noticeable antibodies in the serum by fixation of complement, would start as of the 9th day of illness, according to Drawley and Modern; as of the 13th day or later according to Bengtson. Our experience with cases not only localized in Sicily, but also in other sections of Italy, discovered an important diversity, where precocity of reactions is concerned, depending on the antigen used. With the "Henszlerling" we reached positive reactions in 5 to 6 days from the start of the fever condition. As much as was found in the case of the antigen "Palermo", prepared by us beginning with the rickettsia virus isolated from a patient of our of endemic type and carried into cultivation in a fertilized egg (Duttitta). To us also, the "Nine Mile" antigen, appeared less sensitive and

less specific.

As with Coll, Topping and Coll in parallel research executed with the antigens "Panama", "Mine Hill", "Henzerling", some cases which with "Mine Hill" and with "Henzerling" gave a completely positive reaction, gave negative with "Panama"; thus with us, certain cases during the first days of the illness, had a negative reaction with the "Mine Hill" and positive with "Henzerling", then the separation became even more sensitive with our "Palermo". In a veterinarian, a colleague of ours, sick with a laboratory type and with positive biological proof from the blood in a guinea pig, we obtained a positive complement fixation with the antigen "Palermo" a week ahead of the others.

Caminopetros states that he discovered two goats with milk infection by rickettsia Burneti and a negative fixation of complement in the blood. We never were able to confirm a report of this type, during a vast experience in the field. However, we frequently discovered non-infected milk in animals with a positive complement fixation in the blood, while in all the animals with milk shown to be infected by the biological test we constantly found the fixation of complement in the blood to be positive as also observed by Macbaer and Coll.

According to Bangston, the positivity of the serum agglutinates in magnitude progressively during the first 22 to 25 days of the fever condition; then declines. Our experience also vary in this

case, it has happened, as a matter of fact, to observe patients who did not react positively to the complement fixation during the first days of illness, to react later, in a progressively growing manner. As a matter of fact, we consider, as a general rule at the bed of the patient, a negative serological report during the first days as not eliminating the possibility of Q fever; if it becomes positive during the following days, this represents a truly convincing datum. Thus a positive complement fixation of low type at the start of the illness is considered quite sufficient to evaluate the possibility of Q fever, while on the contrary, now convincing for the diagnosis is a positive reaction of high type during the first days of illness, unless, during the remainder of the course, the type does not give signs of greater increase. As a rule, one should never decide on a diagnosis of rickettsia Burneti on the basis of case of agglutination or of fixation of complement; even though at first it might be of high type it is necessary to ascertain that it will have a tendency to rise during the following days. This is because, in the case of Q fever, as in all rickettsiae, the complement fixation may remain positive long, even months, after the apparent clinical healing (Sulkin and Strauss); and only diminishes slowly and after a long period of time. Thus the danger exists in practical to meet positive fixation of complement, referring not to the active

illness and the origins and nature of which are being investigated but to past infection, clinically healed. The positivity of the sero reaction of which only represents an anamnestic reaction.

In connection with this, one should remember that Denigton found the fixation of complement to be highly positive, in 2 cases, respectively, 305 and 350 days after the start of the fever. We have also observed analogous cases. The greatest persistence found by us in a patient from the start of the illness amounts to 324 days; we were concerned with a nervous form, which continued for more than 3 months with light fever attacks and relapses. The fixation of complement, still appeared highly positive 8 months after all trace of fever and of appreciable nervous changes had disappeared. All our Q fever patients, after leaving the institute, were systematically examined serologically every month, until the complete disappearance of all positivity. Among more common cases, the complement fixation presents a negative reaction after the first examination, it can be stated; a month after the clinical cure, while in cases with persistent pulmonary infiltrates especially in subacute types; in cases with fever recurring in waves; or even with recurring pulmonary episodes; and mainly with changes of the nervous type; the persistence of positive serological reactions beyond the apparent clinical cure is the rule. In view of this, we must ask whether the common explanation given for these facts

can still be held true; that the persistence of antibodies in the blood circulation, detectable by deviations of the complement, represent the expression of an immunity reaction, to be considered on the same level, or on a parallel level with those phenomenon which would lead to a permanent assured immunity after infection by rickettsiae in general and also by Q fever in particular.

DeBorch has been able to prove, during experiments in curing Q fever with aureomycin, frequently and rapidly changes the sero agglutination and the deviation of the complement to negative, even in highly positive cases. In light and non-dangerous cases and mainly with an early start, it is sufficient to administer one dose during the 5 and 6 days of treatment, in medium weight patients, so as to obtain both the disappearance of the fever and the rapid vanishing of the pulmonary infiltrate, and the negation of the sero-agglutinations.

In relapses, of long duration, and of pulmonary changes of less acute duration, the phenomenon unfolds with less evidence, and mainly much more slowly. Even if the fever vanishes after the first administration, or at least drops enough to assume the character of a light fever, the infiltrate is hardly affected, while the nervous and respiratory disturbances are attenuated but not eliminated. In such cases it is necessary to try various (2 or 3) cycles of aureomycin at intervals of a regular number of weeks, so as to observe, the negation of the deviation of the complement, with the disappearance of the clinical factors and mainly of the pulmonary infiltrate.

From my point of view, this observation by DeOrchi is of great importance, especially when tied in with a series of already positively demonstrated facts, i.e.;

- 1) That prolonged and mainly reoccurring forms of Q fever exist, forms which do not comply to the closed cycle, considered as typical of rickettsiae until today. (see for example the dermatophus, both, historical or enclosed);
- 2) That the presence of rickettsia can be proven by the biological test (in the sick men or animals), even long after the vanishing of the fever and the apparent clinical cure;
- 3) That in many cases after the disappearance of fever and the apparent clinical healing, an illness of a type of insistant character and with definite localization, especially pulmonary or nervous, may still cause subjective disturbances of a certain importance (acute asthenia, general ill feeling, important incapacity to functional resumption, etc.) and evident objective phenomena (coughs with expectoration, in the breathing types; violent headache, possibly cold spells, insufficient functional condition mainly of the larynx and pharynx muscles, in nervous forms); subjective and objective disturbances, which sometimes surpass, also in their insistance, the properties of a single convalescence, be it even prolonged.
- 4) That all these phenomenon - meaning the prolonged forms as much as the persistence of both the objective and subjective disturbances,

and the elimination of rickettsia through the urine after decrease of temperature disappear rapidly and definitely if sufficient doses of aureomycin are administered, with the characteristic test such disappearance is definite only when the reaction to the complement fixation is definitely negative.

All these facts lead one to believe that the persistence of a positive reaction to the complement fixation in our patients does not necessarily constitute a phenomenon of anamnestic nature and origin. Referring to cured or regressed infection, but that it shows a phenomenon analogous to that which we and others, Beck and Coll have been able to demonstrate finally; that is, that in certain domestic animals, (horses, bovines, dogs) the rickettsia Darnoti infection may reside in the stage of an inapparent disease. Of the remainder, unless the knowledge in the field of Q fever broadens, unless the number of observed patients with positive complement fixation and with completely negative anamnesis in the sense of having suffered, at a more or less close time of morbid form which can be approximated to an attenuated form of Q fever. (Beck and Coll; Meldolesi; Doodanasaya) but he admitted that Q fever in man, not only may in certain cases unfold as a completely unapparent illness, one may say completely asymptomatic, but it can also pass at a certain moment, from a state apparent illness to a state of sub-clinical illness, so as to simulate a clinical cure. These hypotheses do not contrast in fact with our actual knowledge, which is proved in the fact that Hooper, for dermatophoid, stated in 1929 and proved in 1946 concerning a case of Brill's disease, which appeared spontaneously in Zurich (Hooser and Loeffler) and which had all humoral signs of a

rickettsia Prowazekii; that there exists a possibility, that following the apparent clinical cure, the infection passes to the inapparent(subclinical) state, so as to be able to have, after 27 years of apparent complete health, the resumption of a typical Brill Noddis, without the necessity of any epidemiologic link with some recent case of dermatophoid, therefore, without necessity of reinfection.

In the particular case of Q fever, the persistence of positive serologic reactions long after the apparent clinical cure and the vanishing of all changes in temperature, would indicate in certain particular cases, the passing of the infection to a sub-clinical state. The administration of aureomycin would eliminate the sub-clinical infection; and with final negatization of the sero reactions could lead to the final destruction of the infection and therefore to a true cure.

By these observations we would like to increase the practical value of serological reactions for Q fever diagnosis and to greatly diminish the range of error due to positivity of anamnestic origin.

The proof of rickettsia Burneti in the patient - man or animal - constitutes the more precise search for confirmation, either in the clinical field at the patient's bedside or your experimental research.

The choice animal is the guinea pig, which in cases of positive biological tests, always shows, with rare exceptions, a feverish disease, with characteristic symptoms easy to recognize as

rickettsia Burneti. The guinea pig stands the injection of infected matter in the peritoneum very well, because of which - in the case in which sputum is the material for inoculation - it is conveniently protected from other eventual contemporaneous infections, by an admixture of antibiotics (penicillin 10.000; streptomycin 10 cg).

We have never noticed an evident negative reaction caused by the streptomycin on the development of rickettsia Burneti, as have been observed (Muehner and Coll).

In the clinical and experimental field the blood, urine, sputum and also the milk of the patient may be used as inoculation material. The test has a good chance of success, with anyone of these. In the experimental field sterilized pap of organs can also be inoculated, especially pap of splenic pulp; the latter is exclusively used for infections in series from animal to animal. After 4 to 5 successive days in the guinea pigs, one may attempt a passage into the embryonic chicken egg, following Cox's technique; here, however, research might really meet unexpected difficulties as the adaptability of rickettsia Burneti proves to be different from focus to focus. Among those known and studied today "Nine Mile" proves to be the easiest to cultivate in an egg, while Hemzerring only adapts itself with great difficulty. In our experience, we were successful 5 times, after repeated passing through guinea pigs, in isolating a strain of rickettsia burneti from patients with forms unlike Q fever.

However, only twice did we succeed in transplanting from the guinea pig to the fertile egg, so as to obtain a pure culture, with which, using the technique standardized by Cox we were able to isolate an antigen, which, on our clinical material - human and veterinary - proved to resemble the Hungarian strain very closely, which was proved by Cox, however, with some characteristic differences, with even greater precision. In certain cases, as stated, the complement fixation executed with the antigen derived from the Palermo strain gave a positive result with the same number or less days than it took the antigen of American origin and successively gave much higher results. Everyone of the results arrived at with the antigen of the Palermo strain was in perfect harmony with the results of the biological test in the animal. In the other three cases, however, despite the more scrupulous technique, despite the fact that the eggs showed no sign of defilement; despite the fact that the illness in the animals for experiment had shown all the characteristics of the rickettsia Barnetti infection; despite all this, we were unable to transfer the strain into the embryonic chicken's eggs. A few rare elements of rickettsial aspect were observed during the coloring of the slides with Machiavello's (method), after the first passage, however, during the successive transplantings the eggs remained sterile, even though some gave birth to apparently healthy chicks. In two cases we were concerned with strains obtained from patients originating in different sections of Sicily;

in one case, with a strain isolated from a patient's blood, sent for examination from the Republic of S. Marino, by Dr. Suzzi-Valli.

Some authors (Caporale and Cambacarta) proposed to simplify the biological test, by stopping with first inoculated guinea pig and seeking the rickettsiae on slides from splenic pulp received from the latter, coloring them by Macchiavello's method. We can not accept such a proposal; in the first place, in as much, as by our personal experience, the appearance of rickettsiae in slides of splenic pulp from an inoculated animal, contrary to the affirmations of some authors, mostly American, has been only in exceptional cases and precisely only for inoculation of sputum from patients with edematous broncho-pneumonia, sputum which we know to contain such a highly infectious amount, to result most of the time in the death of the first inoculated guinea pig after 4 to 5 days; in the second place, we do not consider as sufficiently guaranteed, a diagnosis of rickettsia Burneti, based only on a microscopic report of a slide of splenic pulp, colored by Macchiavello's method for, as once occurred to our collaborator, Prof. Mirri and we have also had occasion to note this among other researchers - it is never possible to exclude with complete certitude that, despite the most accurate controls, guinea pigs contaminated with pseudo-tubercular bacilli might enter the stable; indicating the possibility of finding acid resistant elements in the spleen slides, which, when colored by Macchiavello's method, could erroneously be interpreted as being caused by rickettsia Burneti.

In proper circumstances, this biological test in guinea pigs is accomplished with certain ease, so that this method of research can be carried into clinical routine, as a means of research and proof, indispensable in dubious cases, of control in cases of particular interest.

However, for the routine execution of such tests, a particular laboratory arrangement is necessary, lacking which completely false results can be obtained; either all positive or all negative. This is not only limited to the specific competence of the person conducting the observations, the inoculations in the animals and the passing in series from animal to animal and eventually also from the animal to the egg. But beyond this must concern the efficiency of the stable hands, who must guarantee the analyst a strict isolation and a continuous and systematic surveillance of the animals on their arrival and during their stay, so as to be able to have the assurance with every experiment that the animal:

- 1) is not already infected with rickettsia Burneti, in a non-evident form, before the inoculation;
- 2) is not exposed to some type of infection, after inoculation;

Indispensable for this, is:

- 1) that the stable hand have, beyond the usual furnishings necessary for any work of microbiological type, a particular section where the animals after injection can be isolated in special tile lined cement cubicles, separate for every inoculation; that these cubicles guarantee

both complete isolation and the possibility of easy sterilization after every experiment, either by flames or by means of a germicide lamp;

2) that every animal be tested by means of the complement fixation or by sero agglutination both on arrival at the stable and systematically every week, during the entire period preceding the inoculation, so as to make certain that it is not a carrier of a latent rickettsia Burneti infection.

Concerning the consideration of the spread of rickettsia Burneti by zoonosis throughout the animal population, indifferent of the various types; considering the great ease with which the infection spreads from animal to animal; given the enormous frequency, among animals more than among men, of latent infections, completely inapparent, however always able to spread the disease; the possibility of an entire stable becoming infected with Q fever is great. It is sufficient for one animal with an uncontrolled, unapparent infection to enter. Considering the enormous resistance which rickettsia Burneti may demonstrate against common physical agents and common disinfectant, once a stable is infected, it may remain that way for years, and even if detected as such, it is not easily sterilized. An infected stable may lead to colossal errors, well-known cases of which exist in literature, both in the clinical and experimental fields. Furthermore mention has been made of the practice of inoculation of animals and of successive transplantings, for which a particular laboratory set up is also necessary, allowing such maneuvers

to be made sterily under glass hoods, kept sterile by means of ultraviolet germicide lamps.

No particular foresight exists in collecting samples for proof of rickettsia Parviti, except for the common norms of sterility; as far as blood is concerned, we had the best results in using coagulate pup, instead of blood serum. In general, it is preferable to use recent and fresh blood; it is most important that it be obtained sterily and if possible kept in a refrigerator. However, we also had positive results in the biological test in guinea pigs with blood sent by mail in a sterile container. However, according to Combliesco and Dimitresco, the infectious capacity of sterily obtained blood kept at room temperature, in a sterile test tube covered with vasoline oil, remained unchanged for 6 months and 8 days. After this date and up to 9 months and 18 days the infectious quality was not destroyed, but only weakened and that by doubling the amount of blood (by 4cc) the guinea pig became infected, even though only in a non-apparent way, but showing the presence of specific antibodies in the serum, revealed by complement fixation.

Diagnostic.

Diagnosis of Q fever is frequently difficult; often frankly arduous.

It is always based on epidemiological, clinical and laboratory data; among the latter, the radiological and seriological and the biological tests in the animal (guinea pig) are of great importance.

The epidemiological data is always sought out. Before rendering a clinical verdict it is necessary to determine whether a rickettsia

Burneti infection is possible, in general. For this it is necessary, as for any contagious disease, to know the epidemiology of the type perfectly, so as to be able to apply to the study of the single patient, the epidemiological knowledge suggested by the case.

Having past the first difficulty of generic proof of Q fever, the difficulty of ascertaining the particular clinical diagnosis in the case under study remains; difficulty made greater by the multiplicity of clinical fields to which rickettsia Burneti may tend, none of which fields, with the possible exception of edematous pneumonia, has such a precise and definite aspect, so as to be directly isolated, for immediate identification. However one may suspect Q fever, at the patient's bedside, especially if the epidemiology agrees; however, certitude is never possible without the backing of laboratory tests.

Among the laboratory tests, serological reactions (agglutination, fixation of complement) are most important, in the practical field. As we have seen, despite their persisting to positivity long after the apparent cure, the dangerous possibility of error in referring to anamnestic and not active disease conditions because of their positivity in considering them none too important; however, one can not exclude them apriori.

In doubtful cases, one should try the biological test in the guinea pig, which, when definitely positive, gives certain proof of an active infection and can be considered a direct diagnosis.

However, we should clearly define what is to be understood

by direct diagnosis. We may state that if the biologic test gave positive results, the direct diagnosis of the active infection can be considered reached; however, we are not authorized to hold that every lesion, every disturbance shown by the patient under observation, is directly connected, in a casual sense, with the rickettsia Burneti infection, as it could be a case of lesion or of disturbances caused by an illness active before the infection, and which continues its evolution despite the contracted infection.

Also, because of this, in the case of Q fever, the microbiological or serological diagnosis alone, cannot and must not be considered sufficient for a final diagnosis, without the complete explanation and interpretation of the pathologic state of the patient. Also in the case of Q fever, the actual complete clinical diagnosis, must be exclusively guided by the clinical criterion, namely by the weighed consideration of the entire mass of data at our disposal, obtainable both during the direct clinical examination of the patient and from laboratory findings, without excluding the epidemiological contribution; even in lighter and apparently simpler cases, this must be the conclusion of an attentive and observant diagnostically differential deliberation.

Differential Diagnosis.

Usually the diagnosis is much easier in the epidemic forms, in which obvious and acute, even if dangerous, forms are more frequent; while, in the endemic forms or typically long drawn-out, with

attenuated and merged symptoms, light fevers of worn out or unapparent forms.

An acute case of Q fever at the start reminds one, in a general way, of an acute infectious disease and which gives vent to most diverse etiological hypotheses, which then slowly collapse, as the common serological and cultural tests prove to be negative,

From a common influenza attack, the emphasis might be on the rash, the typical movement of the leukocytes, the algae in the motion of the ocular globes; only rapid recovery is lacking, for Q fever always lasts at least 1 to 2 weeks, barring complications. When one can prove the presence of the characteristic pneumonary infiltrate, one must immediately concentrate on the serological microbiological tests and await their positive result, and the successive rise in intensity or in other words, the diagnostic laboratory proof. The more serious forms and more accentuated breathing difficulties, would more likely advance the hypothesis of an atypical pneumonia and above all of a virus pneumonia, which differs from Q fever, given the enormous resemblance down to the radiological character and the localization of the pneumonary infiltrate, is mainly based on the reaction of the agglutination under cold, definitely different in the two types (negative in Q fever), and on the microbiological and serological data, negative in virus pneumonia.

A direct pneumonia could never be confused with Q fever, not even in the central form, given the great diversity of anatomic-pathologic

lesions, of clinical symptomatology, of laboratory reports.

The hypothesis of pneumonia or of secondary broncho-pneumonia will find the best clarification in other concomitant facts. The atypical types of Q fever are almost always difficult to diagnose, because their symptomatology is completely pervaded by the profound and serious toxic chemical character. One will think of Q fever in those cases where the etiology does not find an explanation in the common laboratory tests, especially if, from the start, they resemble phlebotis or acute orchitis (otherwise unexplained). The pneumonary type of rickettsia burneti (edematous, broncho-pneumonia) shows characteristic clinical (a state of great seriousness, intense cyanosis, dyspnea of sometimes asthmatic aspect; acute toxic reaction of pneumonary edema) radiological (micronodular infiltrate, with diffused pneumonary edema) and laboratory reactions, which can lead to a direct diagnosis, especially if confirmed by the cultural reaction of the sputum.

The problems may become difficult when it appears in relapses, especially if as is frequent, each relapse is accompanied by serious breathing difficulties, high fever and hemoptysis, thus reaching the possibility of orchitis (which may be excluded from the relative specific reactions), or even, if during the continuation of the illness, the infiltrate, composed of micronodular foci, should create an evident peribronchitic situation, often taken for a tubercular etiology. In such cases, the laboratory data should be of great value and

precisely the negative search of Koch's bacillus in the sputum and the positive reaction of the rickettsia Burneti both to serological (agglutination, fixation of complement) and even more to the biological reaction in the guinea pig. The differentiation of the pseudo brucellar type, frequently presents difficulty, because it causes the same symptomatology, the analogous epidemiology and mainly the non infrequent possibility of interference between the two types, both in animals and man, so as illustrated best by Mirri. Only the direct certitude of either brucellae or rickettsiae in the blood or the urine would clear up all difficulty.

The problem of light fevers caused by rickettsia Burneti is really difficult; given the negative pneumonary reaction in these patients (lack of infiltrate); considering the lightness of the subjective disturbances, most of the time exclusively borrowed from purely encephalopathic character; given the absence of objective symptoms, exceptive the light swelling of the spleen, which for example, can also be found in healthy subjects, malaria or endemic brucellosis countries. In these cases, one may suspect Q fever only after completely eliminating other diagnostic possibilities, not only by positive serological tests, repeated and agreeing among themselves, but also by means of the biological test in the guinea pig. If reactions of the skin ensue, you will have to extend the research to the possibility of rickettsia Gonori or of rickettsial pox.

One may only think of a nervous type after having ascertained the presence of the pulmonary infiltrate and the positivity of repeated serological reactions (agglutination, fixation of complement). However also in these cases, it would be good to move as cautiously as ever and always to think of the possibility of neurosis and of encephalitis of a different type, infective and toxic. The exquisitely parallel form of the lesions, of a definitely unclear character, limited especially to the bulbar nerves; the characteristic association of simultaneous paralysis of the larynx, pharynx and velopendula which sets in suddenly, without other phenomena of foci or without even the slightest effect on the sensorium and with hardly any fever changes, are all facts which might indicate Q fever. The negative laboratory results, on the basis of the spinal fluid including specific agglutinations and above all the normalcy of the glycorrachia, are data of a certain value. I advise in such cases, before affirming or negating, to always attempt to isolate the rickettsia Burneti from the spinal fluid despite the fact that we have never been successful in this. The rather slow decrease of the type; the fact that the evidence slowly and completely regresses without leaving a trace; its influencibility under therapy, first with aurcomycin and later administering vitamin E; can be useful criterions in a final confirmation of the diagnosis.

In the case of both primitive headaches and those following an active pseudo influenzal form, just as in the presence of nervous and radiological disturbances, the suspicion of Q fever can only be

justified if all the other symptoms, both clinical and laboratory, characteristic of the illness, are present, and especially if the serological reactions and biological test are positive. The latter taken from the blood or better yet from the spinal fluid.

The sub-acute pulmonary infiltrate, especially if atypical by structure and position, always represents a great difficulty to diagnosis, always having to be differentiated from processes of different types, radiologically the same. Each of the three radiological types (peribronchial, circumscribed alveolar, ilo-pneumonic), typical of this form, always give birth to different diagnostic problems, and should be separately considered.

The peribronchial type of the subacute infiltrate has many points of contact, in its radiological aspect, with specifically tubercular or luetical fibrosis; of carcinomatous lymphangitis; of the initial forms of pneumoconiosis and finally, of some chronic circulatory changes.

Basic localization can be of value against tubercular fibrosis, very frequent in Q fever, and the reason for other pulmonary manifestations which can eventually result in a specific pulmonary process.

The lack of simultaneous vascular or mediastinal changes contrasts with this hypothesis of a leucic form.

Carcinomatous lymphangitis always has a diffused character and at the most is accompanied by lesions of the pulmonary flanks.

The changes, both chronic circulatory and from the initial pneumococcosis, never show a definitely interstitial type, as is

characteristic, in this type of rickettsia Burneti infiltrate. The problem of the alveolar circumscribed forms is more difficult; which from a strictly radiological point of view, greatly compare with any pulmonary infiltrate, of any type. The infiltrates connected to circulatory disturbances (multiple or repeated pulmonary infarctions; Miesig's infiltrate; Stark's infiltrate) are all characterized by the simultaneous presence of both diffused pulmonary stasis and of cardiac defect, or of serious alterations of the circulatory functions, noticeable at the general clinical examination.

Among the specific infiltrates, the lentic (pulmonary gumma) as distinguished by the characteristic aspect of both the edges and the contrxture; it is characterized by changes of the same type, affecting other organs and apparatus and especially the large vessels and the mediastinum.

The tubercular infiltrates, both of the Aschmann type and the type surrounding the pulmonary flanks (peri-ilari), constitute a far more complex problem, in desiring to differentiate them from a Burneti infiltrate.

Our experience offers similar cases of infiltrates of uniform opacity, round isolated, with now well defined edges, found in perfectly healthy pulmonary areas at the base, but frequently also subclavcar, with all the characteristics attributed to the Aschmann infiltrate. One must add that frequently in such cases a hemoptysis

occurs at the beginning of the illness, quite similar to a true and proper "alarm hemoptysis", occurring namely, in a serene atmosphere. The temperature may vary from high to low; the velocity of sedimentation is always noticeably high; it may also be a definite lymphocytosis.

In certain cases of this type, the infiltrate acts like a true transient infiltrate. It appears with high fever, slightly depressed general state, dry cough and negative complement fixation. However, in little more than a week the complement fixation becomes definitely positive. The fever falls rapidly, when aureomycin is administered, while the infiltrate shows a rapid tendency to vanish completely and without relapses.

Finally, these transient infiltrates, greatly clarify their pathogenesis by rickettsia Burneti.

In other cases, however, the infiltrate is perhaps accompanied by lighter fever, sometimes also by evening fevers, by very rare dry coughs, by headaches and by some diffused muscular pain. The radiological examination reveals a more dense infiltrate, more evident, of less uniform structure and less fused edges, which shows to further modifications in the following weeks, that is no tendency either to spontaneous evolution, or to forming a central negative picture of the concave type. Considering such behavior, not infrequent for our ascarum infiltrate, one may really advance a suspicion of rickettsia Burneti. Thus one has a clear contrast between certain clinical and

radiological data (especially with the morphological characters of the infiltrate) and the serological and microbiological data; because of this the case is really doubtful. Clarification will result only after the administration of aureomycin. The fever is out of the first series and the reabsorption of the infiltrate begins, a process which however seems decisive only after the second or third series of the antibiotic, that is when the fixation of complement is negative. Thus we have the corroboration of the rickettsial pathogenesis of the infiltrate.

A last group of infiltrates of subacute fever, and sometimes with morphological characteristics not far removed from those of the preceding groups, however, shows a persistence for the same number of weeks, without the least tendency to either spontaneous involution or to excavation; it is in no way affected by aureomycin therapy, even though repeated during three successive series. In all these cases, even despite the definite positivity of the serological test and of the biological test in the guinea pig, the infiltrate must be considered as being of tubercular etiology, and interpret the case as a rickettsia *Burneti* infection, taking place in a patient suffering from active pulmonary lesions.

The ilo-pulmonary forms are also full of diagnostic difficulties. Their radiological picture, varying in different cases, always shows certain morphological analogies to the view of primitive or secondary neoplastic morbid conditions; with infiltrating tubercular forms; with pulmonary localization of the lues or of certain fungi; to

have to consider as very slim the morphological differences offered by radiology, on which to base a differential diagnosis. Here also we are concerned with a general clinical problem and not with a purely radiological argument.

In a case of our experience, the differentiations from a primitive bronchial tumor as really difficult and laborious and was only reached at the price of diligent and protracted observation, with the help of all possible means of research, not excluding bronchoscopy and stratography.

The case was of a middle aged individual, affected for five months by an irregular high (38-39) fever, with quite intense breathing difficulties (dyspnea cough with abundant expectorate, sometimes mixed with blood), also observed by us to be slightly emaciated, and anemic and in a general state of depression. Dense irregular opacity of the flank, the size of a large egg and completely unilateral was discovered radiologically; from which non-ramified liver, irregular in form and size, radiated into the pulmonary region, without being able to call them actual "spider leg" digitations. At the beginning an evident instability of the hemidiaphragm was noted on the affected side, and later it resembled a strong pleuritic reaction of exclusively plastic character. Lesions of the bronchial walls were evident neither during the bronchoscopy nor during the stratography. The serological reactions and the biological blood test gave definitely positive results for rickettsia Burneti. At the administration of aureomycin, in three

Three different doses, the fever vanished rapidly, while the general state improved considerably, so that the patient was soon able to leave his bed for a long convalescence. At the same time, the shadow showed an immediate tendency to decrease, which, however, only happened slowly, so as to be considered definite only after four months. The patient took up his usual occupation completely cured before the year's end. He showed no sign of relapse, while kept under strict ambulatory control. We, therefore, consider justified the affirmation of a rickettsia Barneti pathogenesis, for this neoplastiform ilo-pulmonary infiltration.

As to the possibility of distinguishing subacute infiltrates of Q fever from ilo-pulmonary localization of secondary neoplastics; we quote the case of a 57 year old woman, sick for six months with a definitely undulating high fever with profuse sweating and noteworthy dyspeptic disturbances, but without the least respiratory disturbance. Suspecting a brucellosis, despite negative serological reactions, various cures were attempted, vaccines included, with no result. The patient came to us in a generally poor state of health and anemic; an enlarged liver, but no change in the consistence, which was smooth and painful to palpation. The spleen was slightly enlarged in volume and consistence. The basic laboratory tests were indifferent. The radiological examination negative for the other organs and systems, showed at the thorax a monolateral mass, thick, dense, non-uniform, with irregular edges, clear; simultaneously a thin shadow, irregular, less dense, with fuzzy edges are found in each of the pulmonary fields.

The presence of rickettsia Burneti in the blood and a high positive complement fixation were proven. In view of the obscure nature of the case, an aureomycin cure was started and repeated three times. After the first series, the temperature vanished, while the patient entered into a stage of real recovery. The liver soon resumed its normal size, losing the painfulness. The pulmonary infiltrates had completely vanished after three months; the shadows had also lost all pathological character, in volume and intensity. The patient, continuously kept under observation, is well and has had no relapses of any type.

We have observed cases of differentiation from infiltrant ilo-tubercular forms in three girls with fever for a few months, undernourished and a bad state of anemia and generally, with a high hilar infiltrate, neoplastiform, dense, irregular, lymphocytes separated from the blood, definitely positive. It should be noted that radiographically visible were both the residue of a primary illness, apparently cured, and numerous calcifications, in the hilar area, pointing to cured tubercular activity. Serological examinations and biological tests for rickettsia Burneti were definitely positive. Aureomycin was administered and checked the fever quickly and definitely after the first cycle, while the patients improved in the general state and in strength. The radiologic report showed certain regressive changes in few days after the first series for the use of aureomycin; the complete disappearance is only noticed after a few months and after two complete cycles of aureomycin. By the end of six months all the

patients were cured to confirm the diagnosis, the complement fixation for rickettsia Burneti became negative, simultaneously to the vanishing of the infiltrate.

The distinction from a form of pulmonary lues never offers real difficulty even in the case of serologically positive reactions for lues, which can be observed in the feverish phase of rickettsiae Burneti. The alterations of the mediastinum and of the large vessels were never infected as would be the normal case of tertiary lues.

The last possibility of differential diagnosis concerns pulmonary mycosis and mainly moniliasis, which frequently appears with large amounts of infiltrate, pseudo-tumoral, mono-or bilateral; with pleuritic reactions of a plastic nature, with irregular, constant and often high fever; in a generally upset state; with dyspnea, continuous coughs, abundant expectorate, frequently bloody sputum, the negative results of which, either microscopically or by culture, in favor of a Burneti infiltrate speak; the intradermo reactions of monilia extracts; the examination of the nervous system, which is always characteristically upset during chronic moniliasis.

The serological research, the biological test, the influence of aureomycin are of value as positive data.

In conclusion of this diagnostic study of ours, on subacute infiltrates of rickettsia Burneti, we insist that, in this field, a final decision can only be reached through steady and prolonged clinical examination, not only based on radiological, seriological and microbiological data, but also on a complete examination of the patient.

In this connection, one must not forget the most important fact, that only radiological or just one serological report ~~are~~ never sufficient; that one should always exclude the less infrequent possibilities; that it is always useful to note the therapeutic effect of aureomycin, without being limited to just one administration and mainly giving great weight to the eventual joint report of the regression of the infiltrate and of the negativation of the complement fixation.

358

72